On-Demand Orals

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ON-DEMAND SYMPOSIUM: SINGLE-CELL GENOMICS STEM CELLS 1

IMPLICATIONS OF GOLGI FRAGMENTATION IN HIPSC DERIVED NEURONS

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Aims: Alzheimer’s disease (AD) is the most common cause of dementia, with no current cure. Familial AD (fAD) can be caused by mutations in APP or PSEN1 and PSEN2. Our human induced pluripotent stem cell (hiPSC) model consists of patient hiPSC with mutations in PSEN1 (A79V and P150L) and their respective isogenic controls generated via CRISPR-Cas9 precision gene editing.

Methods: The respective hiPSC have been differentiated into cortical glutamatergic neurons and displayed disease characteristic phenotypes such as increased Abeta secretion, Tau hyperphosphorylation and mitochondrial and synaptic deficits. Intriguingly, these neurons displayed Golgi fragmentation, indicating impairments in protein processing and post-translational modifications. RNA sequencing showed differentially expressed genes involved in glycosylation and glycan patterns.

Results: Such processes take mainly place in the Golgi apparatus, linking Golgi fragmentation and impaired processing. These results were further supported by lectin assays as well as glycan profiling. Golgi fragmentation appears to be an early event in AD pathogenesis, observed prior to mitochondrial and synaptic dysfunctions. Additionally, Golgi fragmentation could be induced in control neurons via Abeta treatment, suggesting a potential cascade of Abeta accumulation, triggering Golgi fragmentation, causing impaired processing of key proteins and metabolites, resulting in neurodegeneration.

Conclusions: Our research highlights the potential of iPSC disease modelling, as well as Golgi processing and glycosylation as possible entry points for AD intervention.
GENERATION OF A COHORT OF FULLY REPROGRAMMED IPSCS AND A CATALOG OF SINGLE CELL QUANTITATIVE TRAIT LOCI IN IPSC-DERIVED NEURONS

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Aims: Reprogram PBMCs from 100 neurologically healthy individuals to induced Pluripotent Stem Cell (iPSC) lines and assess methylation and telomere length to determine the epigenetic age and efficiency of reprogramming. Differentiate these iPSC lines to forebrain neurons and use single cell sequencing to identify cell type specific expression Quantitative Trait Loci (eQTLs) and chromatin accessibility QTLs (caQTLs).

Methods: PBMCs were collected and reprogrammed using the Cytotune 2.0 kit. The resulting iPSCs were checked for pluripotency using pluritest and Taqman hPSC scorecard. DNA methylation was analyzed in donor PBMCs and iPSCs to determine the epigenetic age of each cell type. Absolute telomere length was determined by qPCR. Genotyped iPSC lines were differentiated to forebrain neurons and dissociated at day 60 for single cell RNA and ATAC sequencing.

Results: These iPSCs lines cluster with known stem cells by pluritest and show expression of self-renewal markers alongside downregulation of germ layer markers. DNA methylation in iPSCs shows a complete reset of the epigenetic clock relative to the corresponding PBMCs. Additionally, absolute telomere length is similar across all lines, irrespective of donor age, suggesting full reprogramming. Differentiation of these iPSCs to forebrain neurons results in a population with variable neuronal maturity. Single cell RNA and ATAC sequencing identifies QTLs in specific neuronal subtypes within the population.

Conclusions: We have generated a large cohort of fully reprogrammed iPSCs from neurologically healthy individuals. Single cell sequencing in iPSC-derived neurons identifies cell type specific eQTLs and caQTLs that may help build regulatory maps and prioritize genes at loci associated with neurodegeneration.
Aims: Hematopoietic stem cell transplantation (HSCT) is increasingly being tested as a potential therapy for neurological disorders. The premise of this approach is that HSCT-derived monocytes may infiltrate the brain and differentiate into 'microglia-like' cells. Recent advances in the differentiation of induced pluripotent stem cells (iPSCs) into microglia provide a potential new source of therapeutic cells. However, many questions remain regarding the similarities and differences between human iPSC-microglia and blood monocytes (MNs) following long-term engraftment within the brain.

Methods: To examine the engraftment potential and transcriptional profiles of human monocytes, iPSC-microglia, and their progenitors (iHPCs) following transplantation into the CNS, we developed a novel humanized mouse model lacking the fms-intronic regulatory element of CSF1R (hCSF1RΔFIRE/ΔFIRE). Isogenic sets of iMGs, iHPCs, and blood MNs from three patients were transplanted into the cortex of adult immunodeficient hCSF1RΔFIRE/ΔFIRE mice. Four months later, terminally differentiated human cells were isolated from half-brains and the remaining tissue was processed for immunohistochemistry.

Results: Brain clearing and immunohistology analysis revealed near-complete chimerism of human iMGs and iHPC-derived microglia throughout the brain and spinal cord of hCSF1RΔFIRE/ΔFIRE mice. In contrast, blood MNs exhibit greatly diminished engraftment that is restricted to cortical and hippocampal regions adjacent to the injection site. Furthermore, preliminary analysis has revealed morphological similarities between transplanted iMGs and iHPC-derived microglia, in contrast with transplanted MNs. RNA sequencing studies are ongoing.

Conclusions: Taken together, these results offer novel insights with therapeutic implications for the future development of cell-replacement therapies to address neuropathological disease in the central nervous system.
ON-DEMAND SYMPOSIUM: NEUROPATHOLOGY IN AD, LBD AND OTHER DEMENTIA 1

ELEMENTAL METALLIC NANOPARTICLES IN ALZHEIMER’S BRAINS

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Aims: Altered metal homeostasis has been linked to the development of multiple neurodegenerative disorders including Alzheimer’s disease. However, our knowledge of metal (bio)chemistry in Alzheimer’s lacks the chemical and spatial detail required to understand how metals contribute to disease pathogenesis, preventing the development of effective metal-targeting technologies for disease diagnosis/treatment. Here, synchrotron x-ray microscopy and spectroscopy were used to characterize the nanoscale chemistry of metal inclusions within amyloid plaques from Alzheimer’s brains.

Methods: Amyloid plaques from two Alzheimer’s brains were examined using scanning x-ray fluorescence (XRF) at Diamond Light Source beamline I14, and scanning transmission x-ray microscopy (STXM) at Diamond beamline I08 and Advanced Light Source beamline 11.0.2. XRF maps were collected at 80 nm resolution showing the elemental composition of the plaques. STXM was performed over the copper and iron L-edges at ~50 nm spatial resolution to determine the chemical state of these metals.

Results: Amyloid plaques were found to harbour iron, copper, zinc, nickel, mercury, calcium and potassium at levels detectable by nanofocus XRF. STXM examination of the plaques revealed nanodeposits of elemental metallic Cu⁰ accompanying ferromagnetic elemental Fe⁰, previously undocumented in human biology.

Conclusions: Metallic Cu⁰ and Fe⁰ have distinctly different chemical and magnetic properties from their Cu(I/II) and Fe(II/III) forms in which they are predominately utilized in tissues, and their highly reactive surfaces may generate free radicals and other active species. The discovery of elemental metals in the brain raises new questions regarding their generation and their role in neurochemistry, neurobiology, and the etiology of neurodegenerative disease.
TRANSTHYRETIN INFLUENCES THE CEREBROVASCULAR SYSTEM IN ALZHEIMER’S DISEASE – DECIPHERING THE UNDERLYING PATHWAYS

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Aims: Cerebrovascular dysfunction is common in Alzheimer’s disease (AD), often preceding many of the AD typical hallmarks. Transthyretin (TTR) is a recognized neuroprotective protein in AD, although diminished in the pathology. Here, we aimed to investigate the role of TTR in these vascular alterations and the effect of TTR tetrameric stabilization to improve its function and revert cerebrovascular dysfunction.

Methods: The thickness of the basement membrane (BM) and vessel density were assessed using immunohistochemistry for collagen IV, in AD and non-transgenic (NT) mice with one (TTR+/-) or two (TTR+/+) TTR gene copies. Using a human cerebral vascular endothelial cell line, the effect of TTR on the expression of angiogenic key molecules was evaluated by immunocytochemistry and flow cytometry.

Results: 7-month-old AD/TTR+/- mice showed a thicker BM compared to AD/TTR++ , both in the cortex and hippocampus. Vessel density was reduced in AD/TTR+- only in the hippocampus, suggesting BM thickening precedes the decrease in vessel length. 3-month-old NT/TTR+/- mice also showed increased BM thickness as compared to NT/TTR++ , indicating TTR affects directly the structure of cerebral vasculature. No alterations were detected in vessel length in 3-month-old NT mice, again suggesting that BM thickening precedes vessel length decrease. Mice treated with a TTR tetrameric stabilizer, Iodiflunisal, presented decreased BM thickness and increased vessel length, compared to controls. In vitro, TTR stimulated the expression of angiogenic markers VEGF, Ang-2, IL-6, and IL-8.

Conclusions: Our findings show TTR has a direct impact on the vascular changes that occur in AD and might be used to revert vascular dysfunction.
A NEUROPATHOLOGICAL STUDY OF Aβ AND TAU BURDEN IN BRAIN REGIONS OF POSTERIOR CORTICAL ATROPHY CASES RELATIVE TO TYPICAL ALZHEIMER’S DISEASE.

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Aims: Posterior cortical atrophy (PCA) is a variant of Alzheimer’s disease (AD) which presents with visual and spatial problems, functions attributed to occipito-parietal or “posterior” regions of the brain rather than the memory problems seen in typical Alzheimer’s disease (TAD) and associated with the temporal region. This study aimed to quantify Aβ plaques and tau neurofibrillary tangles (NFTs) in PCA, in comparison with TAD in the frontal, parietal, temporal and occipital brain regions.

Methods: Immunohistochemistry for Aβ and tau was carried out on 26 clinically and pathologically phenotyped PCA cases and 27 age and gender-matched TAD cases.

Results: There was a higher burden of Aβ and tau in the parietal region of PCA compared to TAD. In the PCA compared with the TAD group, there was a significant increase in tau burden in the frontal and parietal regions relative to the temporal regions.

Conclusions: There was a higher burden of Aβ and tau in the parietal region of PCA compared to TAD. In the PCA compared with the TAD group, there was a significant increase in tau burden in frontal and parietal regions relative to temporal regions. The observed higher burden of Aβ and tau in the parietal region of PCA compared to TAD is in line with previous studies. The higher frontal relative to temporal tau burden in PCA is in keeping with the evidence for the frontal region as a particularly vulnerable region for accumulation of tau over time in atypical AD despite a posterior predominant distribution pattern in the occipito-parietal regions at baseline.
ON-DEMAND SYMPOSIUM: NEUROPATHOLOGY IN AD, LBD AND OTHER DEMENTIA 1

PATHOLOGICAL HALLMARKS IN THE RETINA OF PATIENTS WITH FRONTOTEMPORAL LOBAR DEGENERATION

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Aims: Frontotemporal lobar degeneration (FTLD) is a heterogeneous disease, with distinct underlying pathologies that include TDP-43, tau and FUS. Currently no in-vivo biomarkers are available to differentiate these subtypes. The retina, as an extension of the brain, could provide a source of such biomarkers. The aim of this study is to assess the presence of TDP-43 in the retina.

Methods: Post-mortem eyes were collected by the Netherlands Brain Bank from FTLD-TDP(n=4), FTLD-tau(n=4), FTLD-FUS(n=1), AD(n=4), and control donors(n=4). FTLD-TDP cases carried a C9orf72 repeat expansion(n=3) or progranulin mutation(n=1). Eyes were fixed in 4% PFA and dissected in 4 quadrants, embedded in paraffin and cut in 10 µm sections. Immunohistochemical stainings were performed for panTDP-43, phosphorylated TDP-43 (pTDP-43), p62, dipeptides (polyGR/GP/GA) and phosphorylated tau (AT8).

Results: P62 and pTDP-43 immunopositive inclusions are observed at the border of the inner and outer plexiform layer (IPL and OPL). PanTDP-43 shows a nuclear staining. Poly-GR/-GA inclusions are observed in the inner nuclear layer in FTLD-TDP cases. AT8 immunostaining was observed in IPL and/or OPL. p62, pTDP-43 and dipeptide pathology are present in the retina of patients with FTLD-TDP. No p62, pTDP-43 and dipeptide inclusions are present in the retinas of control cases or other FTLD subtypes. Inclusions positive for p62 and pTDP-43 are observed in AD cases.

Conclusions: Manifestations of TDP-43 and dipeptide pathology are present in the retina of patients with FTLD-TDP. With the advances in ocular imaging techniques these findings provide opportunities for a non-invasive retinal biomarker for TDP-43.
ON-DEMAND SYMPOSIUM: NEUROPATHOLOGY IN AD, LBD AND OTHER DEMENTIA 1

STUDY OF HUMAN NEURODEGENERATIVE DISEASES APPLYING SUPER-RESOLUTION MICROSCOPY METHODS

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Aims: Super-resolution (SR) microscopy methods enables the study of the brain on the nanoscale. Our aim is to combine array tomography (AT) and different SR techniques to investigate neuropathological traits that fall under the limits of conventional microscopy.

Methods: We combined AT, a method based on the 3D reconstruction of ultrathin consecutive sections, and Stimulated Emission Depletion (STED) microscopy or Stochastic Optical Reconstruction Microscopy (STORM) in human Alzheimer's disease (AD) and dementia with Lewy bodies (DLB) tissue samples.

Results: Here we show different applications to human neurodegenerative disease. First, we combined AT and STED microscopy to study synaptic p-α-synuclein in dementia with Lewy bodies and reported small aggregates in pre- and post-synaptic compartments. Second, we combined AT and STORM to study single synapses and the pathological accumulation of phosphorylated tau as an early event prior to neurofibrillary tangle (NFT) formation in Alzheimer’s disease (AD). Finally, we studied the nanoscale architecture of human amyloid plaques in AD combining AT and STED microscopy. This technology revealed a dense core with a peripheral halo and provided evidence of higher levels of small Aβ species in autosomal dominant Alzheimer’s disease (ADAD) compared to sporadic AD (SAD) cases.

Conclusions: SR techniques show high potential to unravel pathological traits undetectable using conventional microscopy techniques that may inform about key pathophysiological processes in neurodegenerative diseases.
A NOVEL PROXIMITY LIGATION ASSAY TARGETING PHOSPHORYLATED ALPHA-SYNUCLEIN AND TAU REVEALS PREVIOUSLY UNDETECTED PATHOLOGY ON POST MORTEM BRAIN TISSUE.

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**Aims:** Neurodegenerative diseases (NDDs) including Alzheimer’s disease (AD), Parkinson’s disease (PD), and dementia with Lewy bodies (DLB) display various forms of phosphorylated tau and/or alpha-synuclein (α-syn) aggregates in neuronal or glial cells. However, the conformational and morphological properties of such aggregates have been incompletely characterized. Aim of this study is to investigate the patho-morphological properties of phosphorylated tau and α-syn aggregates with sensitive proximity ligation assay (PLA) technique.

**Methods:** Here, we have exploited the sensitivity and <40 nm resolution of proximity ligation assay, along with the selectivity of antibodies directed against phosphorylated tau and α-syn.

**Results:** Our results indicate that PLA, with antibodies recognizing phosphorylated tau or α-syn epitopes, revealed earlier pathology compared to conventional immunohistochemistry (IHC) on post mortem brain tissue from NDD patients. Apart from displaying classic lesions and dystrophic neurites, punctate neuropil signal, patchy and clustered intra-perikaryal constellations of diffuse cytoplasmic aggregates were detected. Furthermore, we observed unique patho-morphological staining patterns of intercellular protrusions / apical dendrites in adjacent cells that could not be visualized by conventional IHC using the same antibodies.

**Conclusions:** Our novel PLA with a dual phosphorylated antibody approach, can successfully detect earlier pathology on post mortem brain tissues from patients with NDDs.
ON-DEMAND SYMPOSIUM: NEUROPATHOLOGY IN AD, LBD AND OTHER DEMENTIA

ROLE OF PARP1-DEPENDENT CELL DEATH IN NEURODEGENERATION OF PARKINSON’S DISEASE

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Aims: Parkinson’s disease (PD) is the second most common neurodegenerative disorder. During the pathogenesis of PD, α-synuclein (α-syn) assembles into higher ordered structures that ultimately become pathologic and drive neuronal cell death. However, what drives the abnormal assembly of pathologic α-syn and death mechanisms that are activated by pathologic α-syn are not known.

Methods: We previously identified PARP1 activation as a key mediator of pathologic α-syn toxicity and transmission in α-syn preformed fibrils (PFFs) mouse model of PD. We also found Parthanatos-dependent AIF-Associated Nuclease (PAAN), also known as Macrophage migration Inhibitor Factor (MIF) is a member of nucleases that acts as a final executioner in PARP1-dependent cell death. Here we show that neurodegeneration induced by pathologic α-syn occurs via PAAN/MIF nuclease activity using a genetic knockout mouse and a mutant knock-in mouse lacking nuclease activity of PAAN/MIF. We also developed a compound screening system for PAAN/MIF nuclease inhibitors and validated neuroprotective role of a hit compound in vivo.

Results: Genetic depletion of PAAN/MIF and a mutant lacking nuclease activity prevent the loss of dopaminergic neurons and behavioral deficits in the α-syn PFFs mouse model of sporadic PD. Compound screening for PAAN/MIF nuclease inhibitors led to the identification of PAANIB-1, a first-inclass, brain-penetrant PAAN/MIF inhibitor that blocks a critical step in PARP1-dependent cell death. PAANIB-1 protects α-syn PFF-induced neurodegeneration in vivo.

Conclusions: Our findings could have broad relevance in human pathologies where PARP1-dependent cell death plays a role because it opens a new avenue to develop new cell death inhibitors by targeting the druggable PAAN/MIF nuclease.
ON-DEMAND SYMPOSIUM: AD DIAGNOSIS & CLINICAL TRIALS & ADVANCES IN DRUG DEVELOPMENT 1

THE IMPACT OF COMEDICATIONS AND GENOTYPES ON COGNITIVE OUTCOME IN AMYLOID - TAU COMBINATION THERAPIES. A QUANTITATIVE SYSTEMS PHARMACOLOGY STUDY

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Aims: With the approval of Aducanumab, combination trials with tau modulating agents are being considered. While the impact on amyloid biomarkers can be anticipated based on prior clinical experience, the effect on the functional cognitive trajectory is more challenging to predict. In addition, cognitive performance is also affected by comedications and common genotype variants.

Methods: We use a Quantitative Systems Pharmacology (QSP) approach, basically a biophysically realistic computer model of neuronal circuits calibrated with ADAS-Cog from historical trials. The model includes the effect of Ab on glutamate and nicotinic neurotransmission, and of tau oligomers on voltage-gated ion channels. COMTVal156Met, 5-HTTLPR rs23351 and APOE genotype are implemented using human imaging studies. Comedications include standard-of-care AChE-I and memantine, antidepressants and benzodiazepines.

Results: In untreated patients, Tau pathology dominates amyloid pathology, in line with preclinical data and dose-dependently accelerates cognitive worsening with COMT MM having the greatest impact at early stages and COMTVV becoming gradually more important in later stages with a similar profile for 5-HTTs and 5-HTTL. In patients treated with amyloid agents, tau pathology reduces the limited beneficial effect on ADAS-Cog. Virtual patient trials with distributions of comedications and genotypes using the PK profile of aducanumab underscore the importance of the right timing, dosing and stratification criteria for the combination trial. In general, the differential impact of tau pathology on cognitive trajectory decreases with increasing neurodegeneration.

Conclusions: In the absence of actual clinical experience, QSP might be a powerful approach to optimize clinical trial designs for amyloid-tau combinations.
Aims: Longitudinal cognitive testing is essential for developing novel preventive interventions for dementia and Alzheimer's disease; however, the few available tools have significant practice effect and depend on an external evaluator. We developed a self-administered 10-minute at-home test intended for longitudinal cognitive monitoring, Boston Cognitive Assessment or BOCA. The goal of this project was to validate BOCA.

Methods: The BOCA test uses randomly selected non-repeating tasks to minimize practice effects. BOCA evaluates eight cognitive domains: 1) Memory/Immediate Recall, 2) Language Comprehension/Prefrontal Synthesis, 3) Visuospatial Reasoning / Mental rotation, 4) Executive function / Clock Test, 5) Attention, 6) Mental math, 7) Orientation, and 8) Memory/Delayed Recall. BOCA was administered to patients with cognitive impairment (n = 50) and age- and education-matched controls (n = 50).

Results: Test scores were significantly different between patients and controls (p < 0.001) suggesting good discriminative ability. The Cronbach’s alpha was 0.87 implying good internal consistency. BOCA demonstrated strong correlation with Montreal Cognitive Assessment (MOCA) (R = 0.90, p <0.001). The study revealed strong (R=0.94, p <0.001) test-retest reliability of the total BOCA score one week after participants’ initial administration. The practice effect tested by daily BOCA administration over 10 days was insignificant (β=0.03, p=0.74).

Conclusions: The BOCA test has the potential to reduce the cost and improve the quality of longitudinal cognitive tracking essential for testing novel interventions designed to reduce or reverse cognitive aging. BOCA is available online gratis at www.bocatest.org.
ON-DEMAND SYMPOSIUM: AD DIAGNOSIS & CLINICAL TRIALS & ADVANCES IN DRUG DEVELOPMENT 1

COGNITIVE COMPOSITES DOMAIN SCORES PREDICTORS OF CONVERSION TO DEMENTIA IN PATIENTS WITH MILD COGNITIVE IMPAIRMENT.

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Aims: To identify optimal Cognitive Composites (CCs) domain scores related to conversion to dementia from Mild Cognitive Impairment (MCI).

Methods: MCI > 44 years old from the Ace Alzheimer Center cohort (n = 2421) were included. Neuropsychological variables were approached under an exploratory and confirmatory factorial analysis (FA). Three relevant clinical MCI phenotypes were considered: early vs. late onset, amnestic vs. non-amnestic, and probable vs. possible. Cox proportional hazard ratio and Kapplan-Meier analysis were calculated to estimate risk of conversion and survival times to dementia, considering the CCs obtained in confirmatory FA and their interaction with the three clinical phenotypes. The model was controlled by age, sex, and education.

Results: Exploratory and confirmatory FAs supported a five-factor CCs domain scores: Processing Speed, attention and working memory (A-WM), verbal fluency, memory and visuospatial-praxis (V-P). Survival analysis showed that worst CCs domain scores main factors, except for A-WM, with a non-significant association, were associated with a higher risk of conversion. Interaction effects showed that V-P was modulated by early-late onset (Wald=10.93, p=.001, HR=.65) and probable vs. possible conditions (Wald= 6.31, p=.012, HR=1.16). The predictive risk conversion of V-P performance was increased among late-onset and probable patients. Early vs. late-onset and probable vs. possible conditions showed a significant interaction effect (HR= 1.93, p=.021). Accumulation of probable and late conditions in a patient emerges as a critical condition of conversion risk.

Conclusions: Among MCI patients followed in a Memory Unit, CCs domain scores emerge as consistent predictors of conversion to dementia, even when considering other relevant phenotypic variables.
ON-DEMAND SYMPOSIUM: AD DIAGNOSIS & CLINICAL TRIALS & ADVANCES IN DRUG DEVELOPMENT 1

IMPROVED SUVR CALCULATION FOR [18F]-AV45 AMYLOID PET IMAGING USING A NOVEL REFERENCE REGION APPROACH

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Aims: Utilization of a data-driven approach to find an optimal reference region for [18F]-AV45 PET imaging that differentiates the spectrum of Alzheimer’s disease (AD) in both cross-sectional and longitudinal study designs.

Methods: Data of 283 participants (135 amyloid-negative cognitively normal (CN), and 148 amyloid-positive AD) from the ADNI database (http://adni.loni.usc.edu/) was used. All [18F]-AV45 scans were co-registered, normalized, and skull-stripped. The dataset was split into a training-and test-dataset. Voxel-wise group comparisons were performed in the training-set (75 CNs, 77 ADs) to identify a reference region that is void of on-target tracer uptake. Potential clusters were used to extract mean global SUVRs in the test-dataset (60 CNs, 70 ADs). Effect sizes between novel clusters and commonly used reference regions were compared. Baseline and follow-up data of 19 CNs, 36 participants with mild cognitive impairment, and 24 ADs was used to test whether the newly identified cluster is more sensitive to assess longitudinal change than common reference regions. Effect sizes of change in SUVR between baseline and follow-up were used as metric of sensitivity.

Results: The training dataset yielded two novel clusters in the brainstem and the cerebellar white matter. These new reference regions showed higher effect sizes compared with commonly used reference regions. Significant differences in the effect sizes were observed when examining longitudinal change in SUVR computation compared with previously used reference regions.

Conclusions: The data-driven approach using both cross-sectional and longitudinal study designs improved SUVR measurements for [18F]-AV45 imaging. Additionally, longitudinal SUVR quantification benefited from this method, with implications for clinical trial designs.
Mitocondrial DNA Methylation Levels Are Altered in Individuals with Mild Cognitive Impairment

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Aims: Increasing evidence is showing that alterations in mitochondrial epigenetics mechanisms could be involved in the etiology of several human disorders, including specific neurodegenerative diseases. In this regard, recently we observed altered methylation levels of the mitochondrial displacement loop (D-loop) region in peripheral blood of amyotrophic lateral sclerosis patients, but not in individuals with Parkinson’s disease. Regarding Alzheimer’s disease, altered D-loop methylation has been found in brain of patients with AD-related pathology, and a dynamic pattern during the progression of the disease was observed in brain of transgenic AD mice. Moreover, we observed a significant 25% reduction of D-loop methylation levels in peripheral blood of late-onset AD patients when compared to control subjects. However, until now no investigations in peripheral blood of patients with Mild Cognitive Impairment (MCI) has been performed.

Methods: In the current study D-loop methylation levels have been evaluated by means of the Methylation-Sensitive High Resolution Melting in peripheral blood of 18 AD patients, 14 MCI individuals and 105 control subjects. All AD and MCI subjects received a biomarker-based diagnosis.

Results: We observed that D-loop methylation levels were significantly higher in MCI individuals when compared to both AD patients and controls.

Conclusions: Current results, together with our previous data, suggest that D-loop methylation levels change during the progression of AD. More interestingly, D-loop methylation alterations have been detected in peripheral blood, suggesting that further studies deserve to be performed in order to validate the usefulness of D-loop methylation analysis as a peripheral biomarker for early detection and diagnosis of AD.
THE EFFECT OF A MINDFULNESS-BASED VERSUS HEALTH SELF-MANAGEMENT INTERVENTION ON COGNITIVE PERFORMANCE IN OLDER ADULTS WITH SUBJECTIVE COGNITIVE DECLINE (SCD): THE SCD-WELL RANDOMIZED CONTROLLED TRIAL


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Aims: To test the hypothesis that a Caring Mindfulness-Based Approach for Seniors (CMBAS) intervention will confer greater benefit to objective cognition than a Health Self-Management Program (HSMP) in individuals with subjective cognitive decline (SCD).

Methods: This study utilized data from the SCD-Well RCT. Older adults with SCD (n=147) were recruited from memory clinics in four European countries, and randomized to an 8-week non-pharmacological intervention (either CMBAS or HSMP). Objective cognition was assessed at baseline, 8-weeks, and 24-weeks using a battery of tests. Three cognitive composites were also computed - an attention composite, executive composite, and abridged Preclinical Alzheimer’s Cognitive Composite 5 (PACC5Abridged). Linear mixed models estimated the change for each outcome within and between trial arms. All models were adjusted for demographics, baseline Alzheimer’s disease (AD) blood biomarkers (P-tau-181, NfL, and Abeta42), and neuropsychological retest effects.

Results: PACC5Abridged scores increased from week 0-24 in both arms of the trial (p<.001; see figure). The mean increase [95%CI] did not differ between arms (CMBAS=0.32 [0.17, 0.47]; HSMP=0.30 [0.15, 0.45]). Neither the attention nor the executive composite scores increased in either arm. Amongst individual cognitive tests, non-differing improvements were observed in both arms for the DRS-2, RAVLT, and WAIS-IV Coding. Neither trial arm exhibited improvement for verbal fluency, Stroop nor TMT.
**Conclusions:** Scores on a composite sensitive to early AD-related cognitive dysfunction improved in both arms, even after accounting for retest effects. This work adds to the growing body of evidence that non-pharmacological interventions can improve cognition in individuals with SCD, a population at increased dementia risk.
Aims: Hippocampal atrophy occurs early in Alzheimer’s disease (AD). The peripheral blood transcriptome reveals changes in gene expression profiles. The understanding of how the alterations of gene expression relates to hippocampal atrophy is still limited. We leveraged a novel deep learning approach to analyze how transcriptomic changes relate to hippocampal atrophy.

Methods: We used imaging and transcriptomic ADNI data from 426 patients (CN=137, MCI=289), the small size AD group is not used to avoid unbalanced dataset (Table 1). We designed a multi-stage deep learning architecture to learn the hidden patterns of the transcriptomic data. The high-dimensional transcriptomic vector was reduced by K-means clustering and principal component analysis, which selected the representative genes. The generated gene expression vector, along with age and gender information, were then fed into the neural network aiming to predict hippocampal atrophy. The patterns hidden in the high dimensional feature vector were abstracted layer by layer in the network. The learning process is based on the cross entropy loss.

Table 1. Demographic of the dataset

<table>
<thead>
<tr>
<th>Demographic</th>
<th>CN (n=137)</th>
<th>MCI (n=289)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, Mean (SD)</td>
<td>73.11(6.03)</td>
<td>70.88(7.30)</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>64/73</td>
<td>153/136</td>
</tr>
</tbody>
</table>

Results: The dataset was split into 80% and 20% for training and testing purposes. The 49,386-dimension transcriptomic vector was reduced to 3,482-dimension. Using the 3,482 transcripts, our deep learning model achieved an accuracy of 73% and an area under the curve (AUC) of 74% in predicting hippocampal atrophy (Fig.1).
Conclusions: Our novel deep learning approach shows promising results and is expected to greatly advance the application of deep learning in AD research.

Fig1. ROC for hippocampal atrophy prediction
ON-DEMAND SYMPOSIUM: AD DIAGNOSIS & CLINICAL TRIALS & ADVANCES IN DRUG DEVELOPMENT 1

FACTORS GOVERNING RECEPTOR-MEDIATED BRAIN DELIVERY OF BISPECIFIC ANTIBODIES

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Aims: Objectives: Bispecific antibodies targeting amyloid-beta (Aβ) and the transferrin receptor (TfR) could be used for immunotherapy, or when radiolabelled, as positron emission tomography (PET) tracers in in Alzheimer’s Disease (AD). The aim of this study was to investigate in mice how TfR mediated brain delivery of such bispecific antibodies is affected by factors like antibody format, dose, blood cell binding and age of the mice.

Methods: Biodistribution of two formats of iodinated bispecific antibody ligands mAb3D6-scFv8D3 (210 kDa) and di-scFv3D6-8D3 (58 kDa) were investigated ex vivo, in wild type mice. Brain concentration and plasma/blood cell distribution of \[^{125}\text{I}mAb3D6\text{-scFv8D3}\] was compared between young and old, wild type (WT) and AD transgenic (tg-ArcSwe) mice, at two different doses. Capillary depletion and nuclear track emulsion was used to study brain parenchymal-vascular distribution of the antibodies.

Results: The smaller format antibody displayed lower total brain concentration, but had a faster parenchymal delivery, and higher parenchymal-to-vascular concentration ratio. Young WT mice showed a significantly higher total brain concentration 2 h after administration of \[^{125}\text{I}mAb3D6\text{-scFv8D3}\] compared to old mice. At low dosing, \[^{125}\text{I}mAb3D6\text{-scFv8D3}\] was associated with higher distribution to blood cells, and thereby lower plasma concentration, compared to high dosing. At low dosing, plasma concentration was a better predictor of \[^{125}\text{I}mAb3D6\text{-scFv8D3}\] brain delivery, compared to whole blood concentration.

Conclusions: Small format antibodies, with monovalent binding towards TfR have faster parenchymal delivery. At low doses, TfR-antibodies display higher blood cell binding, which could affect brain uptake at early time points in aged mice.
NEUROGENESIS HYPOTHESIS: A CASE STUDY- PHASE 2A CLINICAL TRIALS OF NA-831 FOR THE TREATMENT OF ALZHEIMER'S DISEASE

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Aims: In the hippocampus, new neurons are generated throughout life via a process called adult hippocampal neurogenesis (AHN). In mild cognitive impairment and Alzheimer's disease, AHN is reduced suggesting that augmenting AHN could help prevent or slow cognitive decline. We will discuss the neurogenesis hypothesis and highlight its application as a potential treatment for AD.

Methods: We have developed a small molecule drug NA-831, which activates synaptic AMPA receptors, and increases the expression of BDNF. BDNF is crucial in synaptic plasticity, learning and memory formation in the hippocampus. NA-831 restores neurogenesis by increasing the number of DCX+PCNA+ neuroblast cells. A randomized clinical trial of NA-831 was conducted in 32 patients with MCI, who received 10 mg of NA-831 or placebo orally per day for 24 weeks, and in 24 patients with early AD, who received 30 mg of NA-831 or placebo orally per day for 24 weeks.

Results: After 24 weeks of treatment, NA-831 provided a significant delay in cognitive decline in MCI as measured by ADAS-Cog-13, an average score difference of 3.4 compared to placebo (p = 0.01; ITT). Similarly, NA-831 delayed cognitive decline in early AD, an average score difference of 4.1 compared to placebo (p = 0.001; ITT). CIBIC-Plus showed 78 % of the study participants receiving NA-831 improved (p = 0.01; ITT). NA-831 was well-tolerated at 30 mg/day for 24 weeks, and no serious adverse events were observed.

Conclusions: With the clinical trial results, we will discuss the neurogenesis hypothesis and highlight its application as a potential treatment for AD.
ON-DEMAND SYMPOSIUM: AD DIAGNOSIS & CLINICAL TRIALS & ADVANCES IN DRUG DEVELOPMENT 1

AGE-DEPENDENT EFFECTS OF THE P75 MODULATOR LM11A-31 ON ALZHEIMER’S DISEASE BIOMARKERS IN A SIX-MONTH SAFETY AND EXPLORATORY ENDPOINT TRIAL

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Aims: Basal forebrain cholinergic neurons (BFCNs) degenerate during normal aging and early stages of Alzheimer’s disease (AD). The p75 neurotrophin receptor (p75NTR) is expressed by BFCNs and modulates pro- and anti-apoptotic pathways as well as neurite and neuritic spine integrity. Pharmacological modulation of this receptor with LM11A-31 reverses basal forebrain axonal pathology in mouse models of AD. Older, late-onset AD subjects tend to have greater proportions of mixed etiology dementia while younger AD subjects are considered to have a greater proportion of pure AD pathology. This prompted the examination of age-dependent effects of LM11A-31 in human AD with longitudinal structural magnetic resonance imaging (sMRI) and cerebrospinal fluid (CSF) biomarkers.

Methods: Participants with mild to moderate AD (MMSE score=18-26; age 55-85) were enrolled in a six-month placebo-controlled phase 2a safety and exploratory endpoint trial of LM11A-31. For placebo and drug, age groups were defined using a median-split: younger (<72 years) and older (>=72 years).

Results: Older subjects exhibited smaller BF volumes at baseline than younger subjects in both placebo and drug groups, and exhibited no differences in AD stage according to MMSE score or CSF p-tau/Aβ42 ratio at baseline. Results outlining age-group specific effects of LM11A-31 on longitudinal CSF and neuroimaging biomarkers will be presented.

Conclusions: This investigation points to the possibility that certain biomarkers can demonstrate age-dependency in response to therapeutic interventions in the context of mild-moderate AD. Our results may inform biomarker selection and design of future studies of LM11A-31 in human AD.
ON-DEMAND SYMPOSIUM: AD DIAGNOSIS & CLINICAL TRIALS & ADVANCES IN DRUG DEVELOPMENT 2

DISCOVERY OF A UNIQUE EPITOPE IN THE N-TERMINUS OF PYROGLUTAMATE ABETA FOR USE AS A VACCINE AGAINST ALZHEIMER’S DISEASE

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Aims: Pyroglutamate Abeta (pE3) are abundantly present in the brain of Alzheimer’s disease (AD) patients, form stable oligomers, induce neurodegeneration in mouse models their potential as drug target has not been generally accepted in the past. We discovered a unique epitope in the N-terminus of Abeta pE3-X representing a valid target for active immunization. The therapeutic effect of this vaccine was evaluated using transgenic AD mouse models on the level of amyloid load and glucose metabolism in vivo, memory performance and neuron loss in the hippocampus.

Methods: Transgenic mice (5XFAD, Tg4-42), Morris water maze, neuron count, 18F-Florbetaben-PET/MRI imaging, 18F-FDG-PET/MRI imaging, immunostaining, in vivo amyloid imaging, in vivo glucose uptake

Results: The crystal structures of the binding pocket of TAPAS antibodies revealed that bound N-terminal region of Abeta adopted a novel structure, not related to any fibrillar or amyloid associated conformations reported so far. The crystal structure enabled the design of a novel Abeta peptide epitope for use as a vaccine. Active immunization of the AD mouse model 5XFAD resulted in a striking reduction in amyloid-plaque load in brain tissue by both immunostaining and in vivo 18F-Florbetaben-PET retention analysis. Moreover, using 18F-FDG-PET/MRI imaging we could show a rescue of glucose metabolism after active immunization. In addition, active immunization of the AD mouse model Tg4-42 rescued learning and memory deficits, as well as loss of neurons in the hippocampus. Comparable positive therapeutic indications were also seen after TAP01 antibody passive immunization in both animal models.

Conclusions: The TAPAS epitope is a promising novel vaccination against AD.
Towards a Mechanistic Understanding of Therapeutic Ultrasound as a Treatment Modality for Alzheimer Disease

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Aims: Therapeutic ultrasound is an emerging therapeutic modality that can be used in a scanning mode in combination with intravenously injected microbubbles to achieve transient blood-brain barrier (BBB) opening (SUS+MB) for the delivery of therapeutic agents, or without microbubbles as a neuromodulatory tool (SUSonly). Given that both physiological and pathological ageing (Alzheimer’s disease) lead to a progressive cognitive decline (Götz et al., NatRevNeurosci 2018), the question arises what the therapeutic effects are in Alzheimer’s mouse models and senescent mice and whether the findings can be translated into clinical practice.

Methods: We explored the two ultrasound strategies over a range of ultrasound parameters in amyloid-depositing APP23 mice and senescent wild-type mice, with up to six weekly treatment sessions. Analysis tools included an extensive behavioural, electrophysiological, biochemical, histological, proteomics and imaging analysis.

Results: We will discuss data that reveal that SUS+MB reduces amyloid pathology and restores cognition (Leinenga & Götz, ScienceTranslMed 2015), that BBB opening is required for amyloid clearance (Leinenga et al., BrainResBull 2019) and whether SUSonly is sufficient to restore cognition (in progress). We will further discuss published work (Blackmore et al., MolPsych 2021) and follow-up studies that reveal that both SUS+MB and SUSonly restore LTP deficits and improve cognition in senescent mice via pleiotropic mechanisms including NMDAR-dependent signalling. We will further discuss the development of a clinical-trial ready device as a part of setting up a therapeutic ultrasound platform.

Conclusions: We conclude that therapeutic ultrasound is a non-invasive modality for the treatment for Alzheimer’s disease and other brain diseases.
ULTRASOUND-MEDIATED DELIVERY OF A NOVEL ANTI-TAU ANTIBODY INCREASES BRAIN AND NEURONAL UPTAKE BUT DOES NOT ENHANCE THERAPEUTIC EFFICACY IN K369I TAU TRANSGENIC MICE

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Aims: Tau-specific immunotherapy is an attractive therapeutic strategy for the treatment of Alzheimer’s disease and other tauopathies. However, targeting tau effectively remains a considerable challenge due to the restrictive nature of the blood-brain barrier (BBB), which excludes 99.9% of peripherally administered antibodies. Furthermore, tau is predominantly localised within neurons. We have previously shown that intravenous injection of microbubbles followed by the application of scanning ultrasound (SUS+MB) can transiently open the BBB, allowing up to 19-fold increased IgG antibody uptake by the brain. However, therapeutic efficacy after enhanced brain delivery has not been explored.

Methods: To assess whether ultrasound-mediated delivery of tau-specific IgGs enhances therapeutic efficacy, K369I tau transgenic mice were passively immunised once weekly for 12 weeks with a novel pan-tau antibody, RNF5, in combination with SUS+MB, followed by histological and biochemical analysis.

Results: Treatment with either RNF5 or SUS+MB alone reduced hyperphosphorylated tau. However, the combination treatment (RNF5+SUS+MB), while effective, had no additive effects on tau reduction, despite increased delivery to the brain. This was due to acute neuronal degeneration and inflammation in the areas where RNF5 had extravasated into the brain parenchyma and been internalised into neurons.

Conclusions: Overall, our findings suggest that higher concentrations of IgG in the brain may be detrimental and that the benefit of SUS+MB might reach a threshold such that increased antibody concentration in the brain may become toxic. What renders brain tissue susceptible is currently being investigated. Together, more research is warranted to exploit SUS+MB as a delivery modality.
ON-DEMAND SYMPOSIUM: AD DIAGNOSIS & CLINICAL TRIALS & ADVANCES IN DRUG DEVELOPMENT 2

PERCEPTA: A NEW DIETARY SUPPLEMENT THAT TARGETS BRAIN PLAQUES, TANGLES AND INFLAMMATION - THE TRILOGY OF MEMORY LOSS

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Aims: Brain aging and Alzheimer's disease (AD) demonstrate the accumulation of Aβ containing "plaques" and tau protein "tangles", that contribute to accelerated memory loss and cognitive decline. Neuroinflammation is the 3rd factor contributing to the trilogy of memory loss. We have developed the world's first dietary supplement for the targeting of plaques and tangles in the normal aging brain.

Methods: PTI-00703 cat's claw (Uncaria tomentosa), a specific and natural plant extract from the Amazon rain forest was identified as a potent inhibitor and reducer of both Aβ fibrils and tau protein filaments.

Results: Disaggregation of Aβ fibrils and tau tangles occurred nearly instantly when mixed together. Structural elucidation studies identified constituents within PTI-00703 cat's claw responsible for the Aβ fibril/tau tangle reducing activity to be specific proanthocyanidins (i.e. epicatechin dimers) including proanthocyanidin B2, B4 and C1. Blood-brain-barrier studies indicated that the major components of PTI-00703 cat's claw entered the brain parenchyma within 2 minutes. Proanthocynidin B2 administered to older APP-plaque producing (TASD-41) transgenic mice within 3 months reduced Aβ plaque-load in brain by 58.2%, with memory improvement (i.e. Morris water maze testing) by 57.8% (nearly back to normal as seen in non-transgenic mice). Neuroinflammation (i.e. astrocytosis and microgliosis) was reduced by 69% and 80.3%, respectively. Circular dichroism spectroscopy revealed these polyphenolic molecules (adjacent hydroxy groups on two adjacent aromatic rings) to markedly reduce plaque and tangle β-sheet secondary folding.

Conclusions: Percepta is a recently new dietary supplement product (containing exclusive and patented PTI-00703 cat's claw) now sold in the USA, and soon throughout the world.
Aims: LipiDiDiet is a 6-year, double-blind, parallel-group, multi-center RCT to investigate the effects of the specific multinutrient combination Fortasyn Connect in individuals with prodromal AD / MCI due to AD. We present the study design and discuss the lessons learned.

Methods: The 6-year RCT period was additionally followed by a 2-year open-label trial (total 8 years). Presence of AD was biomarker-validated at baseline in all trial participants.

Results: The trial started in 2008, last visit was in 12/2018. From 311 baseline participants n=100, 59 and 34 opted in for RCT years 4, 5 and 6 respectively. Over the first 36 months of intervention, we observed significantly reduced disease progression on multiple clinically relevant outcomes including function, cognition, memory and brain atrophy. Analysis including years 4-8 is ongoing. The potential non-selective character of the drop-out provides challenges for the regular efficacy analyses. On the other hand, data from the LipiDiDiet trial, one of the longest intervention trials in this population, will provide unique data and shed light on how the trajectories of cognitive decline in the study participants continue over a prolonged period.

Conclusions: Designing a long-term trial amplifies the already existing challenges further, such as bias due to drop-out, participants changing to open-label medication, and ethical considerations of placebo-controlled long-term intervention. Moreover, diagnostic criteria and recommendations on outcome parameters significantly changed during the course of the trial. We will discuss the considerations of an RCT in this population and lessons learned over the years. Funding: EUFP7 No211696 LipiDiDiet

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Aims: Amyloid-beta aggregation is correlated with cognitive decline in Alzheimer’s disease (AD), suggesting the contribution of unfolded protein responses (UPR). However, an alternative to Donepezil that offers similar behavioral improvement and prevents disease progression is not available. Therefore, in this study, we targeted to evaluate the effect of donepezil on UPR related disease pathogenesis.

Methods: The effect of donepezil on AD-related pathological markers (cognition, acetylcholinesterase activity, amyloid-beta aggregation, and tau-hyperphosphorylation) were assessed in rats. Further, the study was expanded to evaluate the effect of donepezil on UPR activation specifically through estimations of translocation of various signaling factors of different cellular compartments (cytosolic, ER membrane, and nuclear). Besides the findings, were correlated with biochemical and histological alterations in rat brain regions (cortex and hippocampus).

Results: Donepezil treatment to rats significantly restores memory impairment and attenuates pathological markers of AD. UPR related signaling factors activated through increased protein level of cytosolic GRP78 in diseased condition which was also significantly inhibited with donepezil treatment. GRP78 dissociation from ER-membrane located transmembrane kinases (PERK & IRE1) and ATF6 caused their dimerization and phosphorylation mediated activation. Such activated kinases lead to activation of signaling cascade involving altered levels of e-IF2α, XBP1, ATF4, TRAF2, NF-kB, JNK, GADD153, caspase12, and caspase3. These diseased conditions related to altered level of signaling factors were attenuated with donepezil treatment and offered partial but significant neuroprotection.

Conclusions: Findings indicate that AD pathology involves the UPRs mediated neuronal death which could be significantly attenuated with donepezil treatment.
ON-DEMAND SYMPOSIUM: AD DIAGNOSIS & CLINICAL TRIALS & ADVANCES IN DRUG DEVELOPMENT 2

SAFETY AND EFFICACY OF GINKGO BILOBA EXTRACT EGB 761® IN PATIENTS WITH MILD TO MODERATE DEMENTIA: RESULTS FROM AN OPEN-LABEL, PHASE IV TRIAL IN INDIA

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Aims: To assess safety and treatment effects of Ginkgo biloba extract EGb 761®, 240 mg daily, in Indian patients with dementia.

Methods: Patients with mild to moderate dementia (major neurocognitive disorder, DSM-5, MMSE 12-24) were enrolled into this open-label, 18-week, phase-IV trial. Safety was assessed by adverse events (AEs), physical examination, ECG, and laboratory tests. Treatment effects were assessed by cognitive testing, behavioral and global rating scales, and analog scales for tinnitus and vertigo.

Results: Safety analyses included 150 patients, aged (mean±sd) 62.8±8.34 years, 43.3% female. During the exposure phase, there were 48 AEs; for 10 of which a causal relationship with the study drug could not be ruled out. There were 2 serious AEs that were obviously due to ongoing diseases and not related to the study drug. There were no AEs of concern and no conspicuous findings in ECG, laboratory tests, or physical examination. For 146 patients, data related to treatment effects were available. All cognitive scores improved significantly during EGb 761® treatment (means±sd): CERAD constructional praxis, +1.2±2.41; recall of constructional praxis, +1.8±2.54; TMT A, -21.6±24.16; TMT B, -27.8±44.78; all p-values < 0.001. There was a significant improvement in behavioral symptoms (BEHAVE-AD, -5.5±5.69, p < 0.001). On the CGI 52.1% of the patients were rated “much improved” or “very much improved”. Improvements were seen in vertigo and tinnitus.

Conclusions: 240 mg Ginkgo biloba extract EGb 761® daily were well tolerated and improved a wide spectrum of cognitive abilities in Indian patients with mild to moderate dementia.
EXPLORATORY IMAGING OUTCOMES OF A PHASE 1B/2A CLINICAL TRIAL OF ALLOPREGNANOLONE AS A REGENERATIVE THERAPEUTIC FOR ALZHEIMER'S DISEASE: STRUCTURAL AND FUNCTIONAL CONNECTIVITY OUTCOMES

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Aims: To report exploratory imaging outcomes of a randomized-controlled, phase 1b/2a multiple ascending dose trial of the regenerative therapeutic allopregnanolone (ALLO), an endogenous neurosteroid, in persons with early Alzheimer’s disease (AD).

Methods: Twenty-four individuals participated in the trial (n=6 placebo; n=18 ALLO) and underwent brain-MRI before and after a 12-week treatment. Hippocampal atrophy rate was determined from volumetric MRI, computed as rate of change, and qualitatively assessed between ALLO and placebo sex, APOEε4 allele, and dose subgroups. White matter microstructural integrity was compared between both groups using fractional and quantitative anisotropy. Changes in local, inter-regional, and network-level functional connectivity were also compared using resting-state functional MRI.

Results: Rate of decline in hippocampal volume was slowed, and in some cases reversed, in the ALLO group compared to placebo. Gain of hippocampal volume was evident only in APOEε4 carriers (range: 0.6-7.8% increased hippocampal volume). Multiple measures of white matter integrity indicated evidence of preserved or improved integrity. ALLO significantly increased fractional anisotropy in 690/690 and quantitative anisotropy in 1416/1888 fiber tracts, located primarily in the corpus callosum, bilateral thalamic radiations, and bilateral corticospinal tracts. Consistent with structural changes, ALLO strengthened local, inter-regional, and network level functional connectivity in AD-vulnerable regions, including the precuneus and posterior cingulate, and network connections between the default mode network and limbic system.

Conclusions: Indicators of regeneration from previous preclinical studies and these exploratory MRI-based outcomes from this small clinical cohort support advancement to a phase 2 proof-of-concept efficacy clinical trial of ALLO as a regenerative therapeutic for mild AD (REGEN-BRAIN© study; NCT04838301).
GLOBAL COMPLEMENT C3 KNOCKDOWN ATTENUATES SYNAPSE LOSS, LONG-TERM POTENTIATION IMPAIRMENT AND ALLEVIATES COGNITIVE DECLINE IN AGED MICE

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Aims: Complement component C3 is an innate, host-defense immune protein involved in synapse elimination. Cerebral C3 is elevated with aging and Alzheimer’s Disease (AD). We reported that germline C3-deficiency protected aged wildtype and APPswe/PS1dE9 mice against hippocampal synapse loss and cognitive decline. Next, we generated a novel C3 inducible conditional knockout mouse line (C3iKO) to allow global C3 lowering at any age.

Methods: We crossed C3fl/fl mice with an inducible, global Cre line (Rosa26-Cre-ERT2+/-) to generate C3iKO mice. Two-to-three month-old C3iKO mice were injected intraperitoneally with tamoxifen (TAM, 75 mg/kg) or corn oil daily for 5 days. Behavioral testing for hippocampal-dependent spatial memory, object memory, and object location was performed at 16-17 months of age. In another study, three-to-four-month-old female mice were injected with either CO/TAM and electrophysiological recording of long-term potentiation (LTP) was conducted in hippocampal slices of TAM-treated and CO-treated mice at 7-8 months of age following incubation of the slices with neurotoxic Aβ S26-dimers.

Results: Serum C3 levels were consistently reduced 85-97% in C3iKO+TAM mice compared to controls. C3iKO+TAM mice performed significantly better than C3iKO+CO-treated mice in all behavioral tasks, indicating that C3 lowering after brain development protected mice from age-related cognitive decline. In the second study, C3 lowering in adult mice protected hippocampal synapses from Aβ S26-dimer-mediated LTP impairment.

Conclusions: Our novel C3iKO mouse model allows for global C3 lowering at any age and will be crossed with AD-like models to evaluate C3 lowering in early-stage AD pathogenesis.
ON-DEMAND SYMPOSIUM: AD DIAGNOSIS & CLINICAL TRIALS & ADVANCES IN DRUG DEVELOPMENT 3

ANALYSIS OF EX VIVO TARGET ENGAGEMENT OF ABETA-OLIGOMER-ELIMINATING COMPOUND RD2 IN BRAIN HOMOGENATES BY SFIDA ASSAY

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Aims: Soluble Abeta-oligomers have been found to be highly neurotoxic and are thought to be responsible for onset and progression of Alzheimer’s disease (AD). Therefore, the elimination of Abeta-oligomers is a promising strategy for therapeutic drug development. In former experiments, compounds that eliminate soluble oligomers of recombinant Abeta in vitro also improved cognition in vivo in transgenic AD mice¹,². Using brain homogenate from transgenic mice and human AD patients as a source for native aggregated Abeta within a complex matrix for screening oligomer-eliminating compounds should predict in vivo efficacy with greater accuracy than a purely in vitro approach alone.

Methods: Abeta-assemblies from brain homogenates are separated according to their particle size by density gradient ultracentrifugation. Brain homogenates as well as multimeric Abeta-species enriched in a certain fraction of brain homogenate are treated with oligomer-eliminating compound RD2 and are then analyzed by the sFIDA assay, a highly sensitive method for detecting and quantitating protein oligomers and aggregates.

Results: An RD2 dose- and incubation time-dependent reduction of Abeta-oligomers in brain homogenates treated with RD2 was observed.

Conclusions: The effect of RD2 on Abeta-assemblies from mouse brain homogenate was in accordance with previous in vitro and in vivo observations. Importantly, Abeta-oligomers in human brain homogenates were eliminated by RD2 as well. Our approach is suitable to predict therapeutic efficacy with higher accuracy than the in vitro approach alone before starting large preclinical animal studies, thus potentially reducing the number of animals used for in vivo tests and enhancing the translational value from preclinical studies to clinical trials.
ON-DEMAND SYMPOSIUM: AD DIAGNOSIS & CLINICAL TRIALS & ADVANCES IN DRUG DEVELOPMENT 3

TREATMENT WITH THE ABCA1 AGONIST CS6253 IN CYNOMOLGUS MONKEYS INCREASES PLASMA APOLIPOPROTEIN E AND Aβ42/40-RATIO

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Aims: Apolipoprotein E (APOE) ε4 genotype, the main genetic late-onset Alzheimer’s disease (AD) risk factor, is characterized by the apoE4 protein having impaired interaction with astrocyte’s ATP-binding cassette transporter A1 (ABCA1) resulting in; Poor cholesterol efflux (Bielicki 2016) to apoE and reduced lipid transport to cells in brain, Build-up of residual cholesterol in lipid rafts, impeding astrocyte function (Rawat 2019), and Increased neuron cell death (Voskuhl 2018). CS6253 is an ABCA1 agonist that in mice pharmacology studies improves cognition in a process where brain apoE protein is unchanged, plasma apoE protein increased and brain Ab42 (and P-tau) reduced. Monkeys as opposed to rodents have Cholesterol Ester Transfer Protein fundamentally affecting distribution of lipids. We hypothesized that CS6253 treatment in monkeys would generate plasma apoE changes with associated effects on Ab42 in plasma and CSF.

Methods: In vivo: Cynomolgus monkeys have 93% homology with human apoE, lipid and amyloid metabolism similar to humans making them representative models for humans. In cynos CS6253 0 (control), 10, and 25 mg/kg iv (groups of 5, 3, 3 per sex) was administered 15 times over 30 days and plasma collected for apoE and Ab42/40-ratio assessments. Analysis: Plasma was analyzed by ELISA for ApoE and Ab42/40 (Simoa).

Results: In both monkey studies CS6253 treatment transiently and significantly increased plasma apoE and Ab42/40-ratio (Figure) from first administration with was fully sustained to the 15th/last dose.

Conclusions: The findings suggests that CS6253 treatment effects in primates can be assessed by monitoring plasma apoE and Ab42/40-ratio levels, to be confirmed in ensuing human studies.
ON-DEMAND SYMPOSIUM: AD DIAGNOSIS & CLINICAL TRIALS & ADVANCES IN DRUG DEVELOPMENT 3

BMD-001, A NANOPARTICLE CONTAINING MIR-485-3P ANTISENSE OLIGONUCLEOTIDE, BLOCKS ALZHEIMER’S DISEASE PROGRESSION

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Aims: Alzheimer’s disease (AD) is a form of dementia characterized by progressive memory decline and cognitive dysfunction. With only one FDA-approved therapy, effective treatment strategies for AD are urgently needed.

Methods: Expression of miR-485-3p was analyzed by real-time PCR in the human frontal cortex (8 healthy controls (HC), 7 AD patients), precentral gyrus (6 HC, 8 AD), cerebrospinal fluid (CSF) (6 HC, 7 AD), plasma exosomes (10 HC, 17 mild cognitive impairment (MCI), 12 AD). To determine the therapeutic potential of miR-485-3p, we investigated whether treatment with a nanoparticle containing miR-485-3p antisense oligonucleotide (ASO), named BMD-001, affected disease progression in 10-month-old 5XFAD mice. BMD-001 was injected into the mice by i.v injection once weekly for four weeks. Behavioral tests were performed at 11 months and their brain pathology was examined after 8-week-washout at 13 months.

Results: We found that the miR-485-3p was overexpressed in brain tissues and CSF of AD patients, and BMD-001 reduced Aβ plaques, tau pathology, inflammasome, neuroinflammation, and cognitive decline in the 5XFAD mice. Furthermore, BMD-001 enhanced Aβ clearance via phagocytosis of Aβ in vitro and in vivo.

Conclusions: Collectively, our findings suggest that miR-485-3p is a useful biomarker as well as a causative factor of the inflammatory pathophysiology of AD. Furthermore, BMD-001 can be a promising candidate for treatment of AD pathology and cognitive decline, establishing a new paradigm in the AD field.
ON-DEMAND SYMPOSIUM: AD DIAGNOSIS & CLINICAL TRIALS & ADVANCES IN DRUG DEVELOPMENT 3

A THREE-STEP PROCESS TO DEFINE PHASE II TRIAL SDESIGN USING A PATIENT ENRICHMENT STRATEGY USING PHASE I DATA AND PUBLIC DATABASES

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Aims: CNS drug development can learn many lessons from the “best practices” used in oncology drug development campaigns.

Methods: The development of XProTM for the treatment of AD used a stepwise oncology-like approach for the drug development. This resulted in a three-step process. First, inclusion criteria were developed to enrich enrollment for AD patients with neuroinflammation (ADi), matching the disease process with the drug MOA.

Results: Neuroinflammation was assessed at 12w by CSF and up to 12m by MRI measure of free water (FW) in white matter AD bundles (IMEKA) with decreases of 15% and 46% respectively. Patients in the trial were compared with patients in the ADNI database with FW data. These patients had less neuroinflammation by FW validating the enrichment strategy. Next step determined impact of ADi on cognitive decline in our target clinical cohort, mild AD. The Phase I patient profile was overlaid on the “rate of progression” to provide an estimate of speed and severity of cognitive deterioration in a group of patients with the clinical profile defined by the Phase I trial.

Conclusions: 1) A patient enrichment strategy using peripheral biomarkers of inflammation resulted in an AD clinical trial population with a high incidence of neuroinflammation. 2) Patients in the ADNI database with neuroinflammation measured by neuroinflammatory biomarkers had more rapid cognitive decline with less variability. 3) Using data from the first two steps, a 6 month, 200 patient biomarker directed, blinded, placebo control Phase II trial was designed. In summary, patient enrichment strategies combined with analysis of public patient databases allows for smaller, shorter Phase II trial designs.
EARLY VISUAL MEMORY LOSS IN AD PATHOLOGY CONTINUUM IS PREDICTED BY SPECIFIC LATERALIZED HIPPOCAMPAL SUBFIELD ATROPHY

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Aims: While hippocampal atrophy is an important contributing factor to memory decline in AD, memory processes are thought to be differentially related to individual hippocampal subfields. The specific role of hippocampal subfields in pre-dementia AD and long-term memory (LTM) functions has not been thoroughly investigated. We aimed at exploring the relationship between hippocampal subfield volumes with AD pathology and LTM symptoms in prodromal AD.

Methods: 105 nondemented right-handed subjects aged 60-84 were selected from the Translational Biomarkers in Aging and Dementia (TRIAD) cohort. Hippocampal segmentation was conducted using the cutting-edge MAGeT-Brain algorithm, extracting lateralized volumes of dentate gyrus and CA4 (DG/CA4), CA2 and CA3 (CA2/CA3), CA1, Strata radiatum, lacunosum and moleculare (SRLM), and Subiculum. Verbal and non-verbal LTM were assessed using Rey auditory verbal learning test (RAVLT) and Aggie figure learning test (AFLT) respectively. AD pathology was detected using amyloid-PET with [18F]AZD4694 and tau-PET with [18F]MK6240. Corrections for age, apoE4 and education were applied.

Results: Non-verbal LTM was significantly correlated with right DG/CA4 and CA2/3 volumes (p<.05 FDR-corrected). Verbal LTM was significantly correlated with bilateral SRLM and CA1 as well as right DG/CA4 volumes (p<.05 FDR-corrected). Specificity and lateralization of non-verbal LTM association with volumes was largely independent from [18F]AZD4694 and [18F]MK6240 uptake.

Conclusions: Specific association of non-verbal LTM with right hippocampal subfield volumes was achieved for the first time. The AFLT might hold promise for studying specific right hippocampal alterations. While hippocampal atrophy was associated with AD biomarkers, its independent prediction of LTM performance suggests that hippocampal subfield volumes offer complementary pathological information.
THE ASSOCIATION BETWEEN REPETITIVE NEGATIVE THINKING AND BLOOD-BASED BIOMARKERS OF STRESS, INFLAMMATION AND NEURODEGENERATION

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Aims: Repetitive negative thinking (RNT) encompasses future-directed (i.e. worry) and past-directed (i.e. brooding) negative thoughts, and has been associated with cognitive decline, amyloid deposition and tau pathology. We aimed to elucidate the mechanism(s) through which RNT may confer increased AD risk, by investigating the association between RNT and blood-based biomarkers of: stress pathway activation (cortisol), inflammation (high sensitivity C-reactive protein [HsCRP] and interleukin-6 [IL-6]) and neurodegeneration (neurofilament light chain [NfL]).

Methods: Baseline data from 135 cognitively intact older adults enrolled in the Age-Well clinical trial was utilised. All participants completed self-report measures assessing worry (Penn State Worry Questionnaire) and brooding (Rumination Response Scale-Brooding subscale) and had blood drawn to allow quantification of cortisol in serum, and HsCRP, IL-6 and NfL in plasma. Associations between worry and brooding and blood-based biomarkers were assessed via a series of adjusted linear (cortisol and NfL) and logistic (HsCRP and IL-6) regressions.

Results: In linear regressions adjusted for age and sex, higher levels of worry (β=0.22, p=0.010) and brooding (β=0.17, p=0.042) were associated with elevated NfL levels (see Figure). The results were unchanged following adjustment for anxiety/depression. No associations were observed between worry or brooding and markers of stress (cortisol: p’s>0.210) or inflammation (HsCRP: p’s>0.146; IL-6: p’s>0.174).

Conclusions: This study provides further support for RNT as an AD risk factor by finding a relationship with NfL, a marker of neurodegeneration. However, no associations were observed with markers of stress or inflammation, thus further research is needed to elucidate the mechanism(s) through which RNT may confer increased AD risk.
ON-DEMAND SYMPOSIUM: AD DIAGNOSIS & CLINICAL TRIALS & ADVANCES IN DRUG DEVELOPMENT 3

SYNAPTIC RESTORATION FOR NEURODEGENERATIVE DISEASE

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Aims: Rigorous autopsy studies during the past 2 decades have indicated that clinical dementia due to neurodegeneration is a consequence of lost synaptic networks in the brain measured at autopsy. Pathophysiologic processes in Alzheimer’s disease (AD) that cause the elimination of synapses and, eventually, the loss of neurons include elevation of A beta oligomers, tau hyperphosphorylation, neuroinflammation, oxidative stress, loss of growth factors such as Brain Derived Neurotrophic Factor (BDNF), and mitochondrial dysfunction through downstream molecular cascades. A paramount therapeutic goal, therefore, is restoration of cognitive function(s) by activating endogenous repairing/regenerating mechanisms that are synaptogenic and anti-apoptotic (preventing neuronal death).

Methods: Bryostatin (cf. Synaptogenix, Inc.) activation of PKC epsilon directly engages the mRNA stabilizing-BDNF-anti-apoptotic pathways with multiple neurotrophic consequences. In extensive pre-clinical studies of Alzheimer’s disease (AD) transgenic mice, bryostatin restored synaptic connections, prevented neuronal death, reduced amyloid plaques, reduced neurofibrillary tangles, reduced oxidative stress, and reduced neuroinflammation (cf. Sun and Alkon, TIPS, 2019).

Results: These neurorestorative efficacies may be responsible for the significant improvement (> 4.0 points) of the Severe Impairment Battery (SIB) scores above baseline (p<.001, pooled) with Bryostatin treatment patients (p= NS for Placebo patients) for advanced AD in two Phase II clinical trials (Alkon et al., 2021, AAIC).

Conclusions: The persistence of the observed clinical improvement for > 30 days after the dosing regimen is consistent with restoration of synaptic networks that may enhance cognitive function in AD as well as other neurodegenerative disorders such as Parkinson’s disease, Fragile X mental retardation, Multiple Sclerosis, and Amyotrophic Lateral Sclerosis.
Aims: TDP-43 is a RNA/DNA binding protein involved in RNA metabolism. Approximately 97% of amyotrophic lateral sclerosis (ALS) and 45% of all frontotemporal lobar degeneration (FTLD) cases have neuronal nuclear clearing or insoluble TDP-43 cytoplasmic aggregates, making this proteinopathy an important target. The loss of nuclear TDP-43 results in the mis-splicing of several genes, including Stathmin-2 (STMN2). Our Aim is to understand the impact of TDP-43 loss on STMN2 expression, localization, and neuronal function and the discovery of therapeutic antisense oligonucleotides in ALS/FTD.

Methods: Human iPSC-derived motor neurons were transfected with a TARDBP ASO to reduce TDP-43 levels. Samples were collected over 21 days for qPCR, Western blot, and immunocytochemistry (ICC) analysis of TDP-43, STMN2, and control Golgi/growth cone markers. To assess functionality, neurite outgrowth was assessed and rescue by therapeutic ASO treatment.

Results: TDP-43 is knocked down leading to STMN2 loss of full-length transcript and protein. The decline in STMN2 protein in the cell body is observed with reduction of STMN2 in the Golgi apparatus and neurites. Antisense oligonucleotides restore STMN2 splicing leading to increased STMN2 positive neurite length, expression of STMN2 in the Golgi, and act as a novel therapeutic intervention for TDP-43 loss of function in ALS and FTD.

Conclusions: TDP-43 pathology is associated with depletion of STMN2 from neurites and leads to golgi structural deficits. Therapeutic ASO treatment restores STMN2 to neurites and Golgi to growth cones suggesting a significant role of STMN2 in the health of human cortical and motor neurons.
Amyloid Pathways and Inflammation: New Evidences from Encephalitis

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Aims: Several investigations argued for a strong relationship between neuroinflammation and amyloid metabolism but it is still unclear whether inflammation exerts a pro-amyloidogenic effect, amplifies the neurotoxic effect of amyloid or is protective. Aim of this study was to evaluate the changes within amyloid, neuronal and glial CSF markers during acute inflammation.

Methods: Forty-two patients with encephalitis and 18 controls underwent an extended CSF panel of inflammatory, amyloid (Aβ40, 42 and 38, sAPP-α, sAPP-β), glial and neuronal biomarkers. Linear and non-linear correlations between CSF biomarkers were evaluated studying conditional independence relationships.

Results: CSF levels of inflammatory cytokines and neuronal/glial markers were higher in ENC compared with controls, whereas the levels of amyloid-related markers did not differ. Inflammatory markers were not associated with amyloid markers but exhibited a correlation with glial and neuronal markers in conditional independence analysis.

Conclusions: By an extensive CSF biomarkers analysis, this study showed that an acute inflammation associated with glial activation and neuronal damage does not impact on amyloid homeostasis.
Aims: Three recent quantitative and highly sensitive white matter (WM) measures from diffusion MRI (dMRI) were analyzed: i- free-water, a marker of neuroinflammation ii- apparent fiber density, a marker of axonal integrity iii- tissue radial diffusivity, a marker of myelin content. Our objective was to characterize these new biomarkers cross-sectionally in ADNI and describe their associations with cognitive outcomes.

Methods: All ADNI cohorts with dMRI were included; 304 NC, 207 MCI and 83 AD analyzed cross-sectionally. From dMRI, free-water, apparent fiber density and tissue radial diffusivity maps were computed in WM bundles altered in AD. Age, sex, apolipoprotein E4 status, intracranial volume and total WM hyperintensities volume were used as covariates.

Results: In AD-bundles, free-water was 53% higher in ADvsNC and 26% higher in MCIvsNC, controlling for all covariates. Baseline cognitive scores were associated to free-water in AD-bundles with a moderate effect size. When the fornix was analyzed individually, the ROC curve from baseline free-water had an excellent accuracy to discriminate AD from NC (AUC= 0.84). Fiber density in the fornix was 14 % lower in ADvsNC and 10% lower in ADvsMCI. Tissue radial diffusivity in the fornix was 5% higher in ADvsHC.

Conclusions: The decline of these white matter imaging measures in the AD spectrum suggest neuroinflammation, axonal deterioration and demyelination, as reported in histological studies. The free-water measure was an excellent classifier of AD patients, which could also allow to select a subpopulation of participants with neuroinflammation in AD clinical trials targeting the immune system.
BLOOD DNA METHYLATION AGE ACCELERATION IN FTD AND PSP: COULD IT BE A USEFUL DIAGNOSTIC BIOMARKER?

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Aims: Frontotemporal dementia (FTD) is the second most common form of early onset dementia. FTD is heterogeneous and encompasses different clinical syndromes. It also overlaps with atypical parkinsonian disorders such as progressive supranuclear palsy (PSP), which also falls into the neuropathological umbrella of frontotemporal lobar degeneration (FTLD). Increasing evidence suggests that DNA methylation plays a key role in ageing and neurodegenerative diseases, including FTD. Fluctuations in DNA methylation levels at specific sites can act as “epigenetic clocks”, from which a biological age may be predicted. We aimed to estimate blood epigenetic age acceleration in FTD, PSP and healthy controls, and investigate whether this could be useful to distinguish these groups.

Methods: We analysed publicly available DNA methylation data from peripheral blood samples of FTD and PSP patients as well as healthy controls (GEO accession number GSE53740). After quality control checks, 351 samples were used for downstream analysis. Epigenetic age was estimated using the Hannum and the Horvath multi-tissue clocks.

Results: When accounting for chronological age and blood cell composition, our analysis revealed an increased epigenetic age acceleration in PSP when compared to FTD as well as to healthy controls. No significant differences were found between FTD and healthy controls after adjusting for chronological age. Both the Hannum and the Horvath clock have shown similar results.

Conclusions: Our results suggest that blood epigenetic age acceleration could be a useful diagnostic biomarker to help distinguishing between PSP and related diseases.
SYNCHROTRON X-RAY SPECTROMICROSCOPY OF IRON DEPOSITS IN NEUROMELANIN-PIGMENTED CELLS OF THE PARKINSON'S DISEASE SUBSTANTIA NIGRA

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Aims: Neuromelanin-pigmented neurons in the Parkinson’s disease substantia nigra are known to harbour elevated levels of iron in the diseased state, and this has been implicated in their known vulnerability. Neuromelanin is a principal intracellular binding site for iron and other metal elements, and so the primary objective of this study was to determine if iron foci independent of neuromelanin were also present in these cells.

Methods: Characterisation of intracellular metal ion deposits and their surroundings requires a unique balance of spatial resolution, sensitivity, and specificity, crucially without disrupting the native tissue chemistry. Synchrotron x-ray spectromicroscopy was used for this purpose. Post-mortem fresh-frozen substantia nigra from three Parkinson’s disease cases were resin embedded, ultra-microtome-sectioned and analysed using scanning transmission x-ray microscopy (STXM) at the carbon and oxygen K-edges, and the iron L-edge, using the I08 beamline, Diamond Light Source synchrotron (UK).

Results: Neuromelanin was imaged by STXM with our recently discovered method that utilizes a unique feature in the neuromelanin x-ray absorption spectrum at 287.4 eV for label-free visualisation of neuromelanin. A range of oxidation states were revealed for neuromelanin-associated iron deposits on sub-micron length scales in Parkinson’s disease substantia nigra. Iron-rich inclusions independent of the neuromelanin were also identified and characterized. These included examples of ferric iron inclusions within cell nuclei.

Conclusions: This non-destructive label-free imaging of the organic and inorganic components of human brain tissue delivered unprecedented sensitivity at sub-cellular resolutions. Here we present new evidence of iron inclusions within the nuclei of neuromelanin-pigmented neurons in Parkinson’s disease.
Aims: Covert stroke is one underlying comorbidity associated with dementia, as stroke survivors are more than twice as likely to develop Alzheimer’s disease (AD). The ischemic cascade may initiate neuropathological changes, leading to an increased susceptibility for AD. However, due to the long prodromal phase of AD in humans, this has been a challenging endeavor to accurately model in preclinical models.

Methods: The endothelin-1 (ET1) model of ischemia was used in TgF344-AD rats, which exhibit age-dependent cerebral amyloidosis, CAA, tau pathology, neuronal loss, and cognitive dysfunction. TgF344-AD and non-transgenic littermates (nTg) underwent two stereotaxic ET1 or sham (vehicle) surgeries to the medial prefrontal cortex. Neurological deficit was assessed before and after surgery, and structural MRI one week following surgery. Immunofluorescence staining was conducted to examine amyloid load in the cerebral vasculature, as well as changes in the neuroglia.

Results: MRI analysis confirmed the presence of subclinical ischemic events, without changes in neurological deficit scores as a result of ET1 surgery, nor between TgF344-AD and nTg rats. After aging, ET1-treated TgF344-AD animals showed a localized increase in CAA in comparison to sham TgF344-AD animals. Further regional changes in myelin, microglia and astrocytes were observed as a result of ET1 strokes.

Conclusions: Our results demonstrate that focal subclinical ischemia prior to AD onset in TgF344-AD rats resulted in regional changes proximal to the location of the focal ischemia.
ON-DEMAND SYMPOSIUM: NEUROPATHOLOGY VASCULAR SYSTEM, INFLAMMATION, MITOCHONDRIA, DIAGNOSTICS, AD, PD, PSP AND RELATED DISEASES

PROTEOMIC BIOMARKERS, NEUROQUANT DATA, AND SYMPTOMS OF BRAIN NEURONAL INJURY IN ADULTS WITH SUBJECTIVE COGNITIVE IMPAIRMENT

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Aims: a) Examine correlations among brain MRI with NeuroQuant® data and proteomic biomarkers (TGFβ1, MMP9, VEGF, and C4a) in adults with SCI. b) Examine correlations among brain MRI with NeuroQuant® data and symptoms (fatigue, poor focus and concentration, poor memory, poor assimilation of new knowledge, word recollection difficulties, and confusion) in adults with SCI. c) Examine correlations among sociodemographic data (age, gender, ethnicity, and race) and proteomic biomarkers (C4a, MMP-9, TGFβ1, and VEGF), and brain MRI with NeuroQuant® data in patients with SCI compared to age-matched controls.

Methods: A retrospective study design using secondary data analysis was used to examine the interrelationships among NeuroQuant® data, symptoms, and proteomic biomarkers in patients with SCI. Aged matched controls were used for NeuroQuant® data, symptom and proteomic data.

Results: There were eight areas of the brain significantly related to levels of Complement 4a (C4a): forebrain, lateral ventricles, inferior lateral ventricles, hippocampus, thalamus, and pallidum. Increased serum TGFβ-1 levels were significantly associated with increased hippocampus and caudate volume. There were no significant correlations between MMP9 and any brain area volumes. Increased serum VEGF levels were significantly associated with increased hippocampus volume and decreased amygdala volume.

Conclusions: The central hypothesis was that there would be positive increase in proteomic plasma biomarkers’ levels with volumetric changes observed in the brain MRI with NeuroQuant® data in adults with SCI. Atrophy to the forebrain, lateral ventricles, hippocampus, and amygdala was observed in the presence of upregulated C4a, TGFβ1, and VEGF. There was a positive correlation between SCI symptoms and volumetric atrophy to the brain, specifically the hippocampus and cerebellum.
ISLET AMYLOID POLYPEPTIDE IS LINKED TO VASCULOPATHY IN PATIENTS WITH ALZHEIMER DISEASE

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Aims: Studies suggest that aggregated pancreas-derived islet amyloid polypeptide (IAPP - also called amylin) crosses the blood-brain barrier (BBB) and deposits together with amyloid-beta (Aβ) in the brain vessel walls of patients with Alzheimer’s disease (AD). Our objective has been to investigate how aggregated, and thereby biologically inactive IAPP affects the BBB and whether the peptide is implicated in the vascular changes/pericyte loss seen in AD patients.

Methods: The presence of IAPP in relation to pericytes, BBB permeability, and AD biomarkers in human hippocampal and retina tissue, CSF, and plasma were analyzed using immunohistological stainings and immunoassays. Experimental in vitro studies on cultured human pericytes were used to study the direct impact of oligomer IAPP.

Results: We have shown specific IAPP inclusions within pericytes demonstrating apoptotic features. Moreover, the number of retinal pericytes was associated with biologically active IAPP in AD patients, while retinal levels of aggregated IAPP correlated with aggregated Aβ levels and with hippocampal IAPP levels. We’ve also found correlations between plasma IAPP levels and CSF AD biomarkers as well as Q-albumin. Our experimental studies demonstrate a detrimental impact of oligomeric IAPP on pericyte viability and autophagy.

Conclusions: Our studies show that IAPP accumulates in pericytes, which causes autophagy disruption and cell death. They also suggest that IAPP accumulates together with Aβ in the retina and that this accumulation is related to brain IAPP accumulation. These findings, along with the found relationship between IAPP levels, AD pathology, and BBB permeability, support the suggested role of IAPP in AD.
CEREBROVASCULAR AND NEURONAL DYSFUNCTION IN THE EARLY STAGE OF ALZHEIMER'S DISEASE

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Aims: This work aims to characterize alterations in cerebrovascular and neuronal function in early stage of Alzheimer’s disease (AD) in terms of resting perfusion and cerebrovascular reactivity to stimulation using a transgenic rat model of AD. The MR-based imaging protocol in combination with robust physiological challenge paves the way for clinical implementation of imaging-based risk assessment of AD in patients.

Methods: Nine-month-old TgF344-AD (TgAD), a rat model of early stage AD with manifestation of AD hallmark pathologies, and age-matched homozygous non-transgenic (nTg) rats were studied using the pseudo-continuous arterial spin labeling MRI. Resting perfusion and hemodynamic responses to 10% CO₂ challenge and forepaw electric stimulation were measured for assessment of cerebrovascular reserve and neuronal function.

Results: While the resting perfusion was not distinguishable between TgAD and nTg rats, TgAD rats had 49% and 58% attenuation in global and hippocampal vascular reactivity compared to those in nTg rats. Further, the hippocampal response area was 50% lower in TgAD rats. Vascular reactivity in the forelimb region of the primary somatosensory cortex (S1FL) was 69% lower in TgAD rats than in nTg rats. While the S1FL activation area was not different, global and cortical activation areas were lower by 65% and 63%, respectively, compared to those in nTg rats.

Conclusions: There is a significant alteration in cerebrovascular and neuronal function that precedes the AD’s clinical symptoms, corroborating the findings in AD patients. Also, this study reinforces the clinical potential of MR imaging as a viable option for early detection of AD-associated neuropathologies for development of early interventions.
LINKING ALPHA-SYNUCLEIN AGGREGATION AND MITOCHONDRIA

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Aims: Alpha-synuclein aggregation and mitochondria are both involved in Parkinson’s disease, but the link between the two is not clear. Here we aim to study this connection in an unbiased, label-free, proteome-wide manner.

Methods: We apply the Limited proteolysis coupled mass spectrometry (LiP-MS) method to characterize cells infected with aggregated alpha-synuclein particles. LiP-MS, newly developed in our laboratory, allows detection of structural changes and altered protein-protein interactions of thousands of proteins at the same time directly from cell lysate in their native environment with a resolution of ~10-20 aa. Therefore, LiP-MS gives a new layer of information invisible for other omics methods because LiP-MS detects far beyond changes in protein abundance.

Results: Here we first expose live SH-SY5Y cells to fragmented fibrils of alpha-synuclein for a long time and observe the global response of cells to the exogenous alpha-synuclein fibrils and distinguish the altered cellular pathways. Second, we exposed SH-SY5Y lysate shortly to the same alpha-synuclein fibrils to detect direct interactors of alpha-synuclein. By crossing the lists of significantly altered proteins and fibrils binders, we could distinguish direct interactors from proteins indirectly affected by alpha-synuclein aggregates.

Conclusions: In our unbiased screen, we detected, among others, statistical enrichment of mitochondrial proteins. The majority of these proteins localize in the intermembrane space of mitochondria or the inner and outer mitochondrial membranes. Moreover, many proteins that are genetic risks of Parkinson’s disease were identified as direct or indirect interactors of alpha-synuclein fibril.
Aims: The ongoing Phase 2, open-label, PARADIGM Study (NCT04476017; 718-CNP-201) evaluates SAGE-718 3mg once-daily in participants with mild cognitive impairment due to Parkinson’s Disease (PD-MCI).

Methods: PD patients, aged 50-75 years, meeting PD-MCI criteria (Movement Disorders Society Task Force) and baseline Montreal Cognitive Assessment (MoCA) score of 20-25 were administered SAGE-718 3mg tablets once-daily for 14 days. The primary endpoint was safety; pharmacokinetics, cognitive performance, and motor symptoms were also evaluated.

Results: Eleven patients were enrolled, mean (SD) age was 69.1 (6.9) years, mean (SD) MoCA baseline score was 23.8 (2.3), all patients were white, 91% Hoehn & Yahr stage 2, and 82% male. Using a comprehensive battery of tests to assess multiple domains of cognitive performance, SAGE-718 was associated with improved performance at Day 14, compared to baseline, on tests of executive functioning (multitasking, stockings of Cambridge, spatial working memory, digital symbol substitution, and two-back test) and an emerging signal suggests improved performance on tests of learning and memory (paired associates, pattern recognition, and verbal memory). No appreciable effect was observed on measures of simple attention/psychomotor speed. No severe/serious adverse events (AEs) or deaths were reported, and no treatment-emergent AEs were considered related to study drug or resulted in study drug discontinuation or study withdrawal. Plasma concentration data suggest a pharmacokinetic profile consistent with previous studies.

Conclusions: These data support the emerging clinical profile of SAGE-718 in PD-MCI, suggesting improved performance on executive functioning, as well as promising signals on learning and memory. A 4-week dosing cohort is ongoing.
A RANDOMIZED FIRST-IN-HUMAN STUDY WITH UB-312, A UBITH® ALPHA-SYNUCLEIN PEPTIDE VACCINE

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¹Vaxxinity Inc, Clinical Development, Dallas, United States of America, ²Centre for Human Drug Research, Neurology, Leiden, Netherlands, ³Vaxxinity Inc, R&d, Dallas, United States of America

Aims: Alpha-synuclein (aSyn) is believed to play a central role in Parkinson’s disease and is considered a target for disease modification. UB-312 is a synthetic aSyn peptide conjugated to a T-helper peptide and is expected to induce antibodies specifically against pathological aSyn, making UB-312 a potential immunotherapeutic for synucleopathies. The UB-312-101 study is aimed to investigate the safety, tolerability, and immunogenicity of UB-312 vaccination in healthy participants, and to determine a safe and immunologically optimal dose for the first-in-patient study.

Methods: 50 eligible healthy participants were enrolled in a 44-week, randomized, placebo-controlled, double-blind study. Participants in 7 cohorts were randomized to 3 intramuscular UB-312 (doses ranging between 40-2000 µg per injection) or placebo injections in Weeks 1, 5 and 13. Safety and tolerability were assessed by adverse events, clinical laboratory, vital signs, electrocardiograms, and neurological and physical examinations. Immunogenicity was assessed by measuring serum and cerebrospinal fluid anti-aSyn antibody concentrations.

Results: Of the 50 participants randomized, 23 participants received all 3 vaccinations up to 300 µg of UB-312. Most adverse events (AEs) were mild, transient, and self-resolving. Common treatment-emergent AEs included headache, nasopharyngitis, vaccination site pain, lumbar puncture site pain and fatigue. UB-312 induced dose- and time-dependent antibody production. Antibodies were detectable in serum and CSF of all participants receiving the 300/300/300 µg UB-312 dose regimen. The average CSF:serum ratio was 0.2%.

Conclusions: UB-312 was generally safe, well tolerated and induced anti-alpha-synuclein antibodies in serum and CSF of healthy participants. The 100 and 300 µg doses are selected for further evaluation in Parkinson’s disease participants.
Aims: We previously discovered that a small (3mm diameter) magnet attached to rat skull at bregma inhibits the dopaminergic neuronal damage in substantia nigra (SN) caused by intraventricularly administered 6-hydroxydopamine (6-OHDA). As neurotrophic factors (NTFs) deficiency and neuroplasticity dysregulation are implicated in neurodegenerative diseases including Parkinson’s disease we examined SMF’s effects on protein levels of NTFs and structural neuroplasticity-related proteins (SNRP) in cortex, striatum and ventral midbrain of rats following implantation of the small magnet.

Methods: Sprague-Dawley rats (300g) were anesthetised with isoflurane and local anesthesia with lidocaine/bupivacaine and a small disc-shaped permanent magnet (inducing 2-5 Gauss SMF intensity at the SN), was fixed to the skull, or a same-sized non-magnetic disc in controls. One, three or five weeks later, the animals were euthanized and levels of target proteins in brain areas were determined by western blot.

Results: The time course of protein changes varied with the individual proteins. At the 5-week time point, SMF-treated rats showed enhanced protein levels of NTFs, including brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), and structural neuroplasticity-related proteins, including synapsin, synapsin-1, synaptophysin, growth associated protein 43 and postsynaptic density protein 95, in the ventral midbrain.

Conclusions: Application of SMF to skull enhances NTF levels in cortex and ventral midbrain. Upregulation of NTFs and SNRP may be the cause of the neuroprotective effect of SMF. Because application of magnetic fields is a non-invasive, cheap and mobile treatment, SMF has potential use in treatment of neurodegenerative disorders.
CLAUDIN-5 BINDER ENHANCES FOCUSED ULTRASOUND-MEDIATED OPENING IN AN IN VITRO BLOOD-BRAIN BARRIER MODEL

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Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, University of Queensland, Clem Jones Centre For Ageing Dementia Research, Brisbane, Australia

Aims: The blood-brain barrier (BBB) is a major obstacle for brain diseases. It can be overcome by combining focused ultrasound (FUS) with microbubbles (FUS+MB). To lower the potential risk associated with high acoustic pressure, we explored preincubation with a claudin-5 binder to lower this threshold.

Methods: We generated a stable MDCK II cell line (eGFP-hCldn5-MDCK II) that expresses fluorescently tagged human claudin-5. Two claudin-5 binders, mC5C2 (a peptide) and cCPEm (a truncated form of an enterotoxin) were synthesized and assessed for their abilities to enhance the permeability of cellular monolayers. We then performed a comparative analysis of single and combination treatments.

Results: The novel cell line formed functional monolayers as validated by an increased transendothelial electrical resistance (TEER) reading and a low (< 0.2%) permeability to sodium fluorescein (376 Da). The two binders exerted a time- and concentration-dependent effect on BBB opening when incubated over an extended period, whereas FUS+MB caused a rapid barrier opening followed by recovery after 12 hours within the tested pressure range. Importantly, preincubation with cCPEm prior to FUS+MB treatment resulted in greater barrier opening compared to either FUS+MB or cCPEm alone as measured by reduced TEER values and an increased permeability to fluorescently labelled 40 kDa dextran (FD40).

Conclusions: The data suggest that pre-incubation with clinically suitable binders to BBB tight junction proteins may be a general strategy to facilitate safer and more effective ultrasound-mediated BBB opening in cellular and animal systems and potentially also for the treatment of human diseases of the brain.
Aims: The aim of our work is to enhance lysosomal function in order to decrease α-synuclein aggregation in neuronal cells as well as in a mouse model harbouring a lysosomal deficiency. We postulate that α-synuclein degradation can be enhanced by the application of human recombinant lysosomal enzymes.

Methods: We treat α-synuclein-aggregating cell and mouse models with recombinant lysosomal enzymes. As models we apply: 1) Cell models with α-synuclein overexpression 2) iPSC-derived dopaminergic neurons carrying an α-synuclein mutation (A53T). 3) Cathepsin-deficient mouse model exhibiting α-synuclein aggregation. As readouts we apply biochemical and microscopic analyses to validate lysosomal function and aggregated α-synuclein pathology.

Results: Our experiments reveal that recombinant lysosomal enzymes are efficiently endocytosed by neuronal cells where they are correctly targeted to lysosomes and matured to enzymatically active proteases. In cell lines as well as dopaminergic neurons derived from iPSCs of Parkinson disease patients harboring mutations within the α-synuclein (SNCA) gene, intracellular enzyme function was increased and α-synuclein levels were reduced after treatment with the recombinant enzyme. Additionally, we observed a decrease of aggregated α-synuclein in the brain and primary neurons of a cathepsin-deficient mouse model exhibiting α-synuclein pathology. Moreover, we demonstrate an overall improvement of lysosomal and cellular function after reduction of pathological α-synuclein by treatment with lysosomal enzymes.

Conclusions: Our findings indicate that lysosomal (dys)function is critical for α-synuclein aggregation as well as clearance. Thus, enzyme replacement strategies utilizing recombinant enzymes have shown to decrease intracellular α-synuclein burden and may be of therapeutic interest for Parkinson's disease and other α-synucleinopathies.
ON-DEMAND SYMPOSIUM: PD, LBD DIAGNOSIS & CLINICAL TRIALS & ADVANCES IN TRANSLATIONAL DRUG DEVELOPMENT

CLINICAL CHARACTERISTICS IN PATIENTS WITH PARKINSONISM ACCORDING TO THE PATTERNS OF DOPAMINE TRANSPORTER IMAGING

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Aims: In this study, we investigated the clinical characteristics of three groups (normal finding; atypical finding of Parkinsonism; typical finding of Parkinson’s disease [PD]), which were differentiated based on the pattern of [¹⁸F]FP-CIT PET/CT uptake.

Methods: We retrospectively evaluated patients with clinical parkinsonism who underwent brain [¹⁸F]FP-CIT PET/CT scan from June 2020 to June 2021. Based on the imaging results, total number of 90 patients were selected and classified into three groups of each 30 patients: normal finding, atypical finding of parkinsonism, and typical finding of PD (Figure). To compare the clinical characteristics between the three groups, data were collected from the medical records near the scan date. The included data were sex, age, BMI, symptom duration, and the presence of hypertension, diabetes mellitus, gait disturbance, tremor, rigidity, bradykinesia, symmetricity of symptom, hyposmia or anosmia, constipation, RBD, cognitive decline, and depression. The clinical features of each group were explored using one-way ANOVA followed by post-hoc Tukey or Games-Howell test.

Results:
Between the three groups, frequencies of tremor (p=0.048), bradykinesia (p=0.006), constipation (p=0.019), and RBD (p=0.014) were statistically different (Table). Tremor was more frequent in normal group than in aPD group (p=0.05). Bradykinesia was more frequently observed in aPD group (p=0.011) or PD group (p=0.023) than in normal group. Constipation was statistically frequent in PD group than in aPD group (p=0.016). The frequency of RBD was higher in normal group or PD group than in aPD group with no significant difference (p=0.061).

**Conclusions:** Significant differences between the groups may suggest potential clinical features of patients with parkinsonism on predicting the nigrostriatal dopaminergic status.

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Atypical PD</th>
<th>PD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number, n</strong></td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>-</td>
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<tr>
<td><strong>Sex (female/male)</strong></td>
<td>21/9</td>
<td>13/17</td>
<td>20/10</td>
<td>0.072</td>
</tr>
<tr>
<td><strong>Age (years, mean ± SD)</strong></td>
<td>72.1 ± 11.5</td>
<td>74.7 ± 9.4</td>
<td>71.7 ± 9.3</td>
<td>0.476</td>
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<tr>
<td><strong>BMI (kg/m², mean ± SD)</strong></td>
<td>25.1 ± 3.5 (n=20)</td>
<td>23.4 ± 3.6 (n=11)</td>
<td>24.9 ± 4.1 (n=24)</td>
<td>0.487</td>
</tr>
<tr>
<td><strong>Duration of symptom onset (months, mean ± SD)</strong></td>
<td>29.7 ± 50.2</td>
<td>19.7 ± 27.1</td>
<td>14.0 ± 17.9</td>
<td>0.230</td>
</tr>
<tr>
<td><strong>Hypertension, n (%)</strong></td>
<td>9 (30.0)</td>
<td>16 (53.3)</td>
<td>17 (56.7)</td>
<td>0.074</td>
</tr>
<tr>
<td><strong>Diabetes mellitus, n (%)</strong></td>
<td>3 (10.0)</td>
<td>3 (10.0)</td>
<td>3 (10.0)</td>
<td>1.00</td>
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<tr>
<td><strong>Gait disturbance, n (%)</strong></td>
<td>19 (63.3)</td>
<td>25 (83.3)</td>
<td>18 (60.0)</td>
<td>0.080</td>
</tr>
<tr>
<td><strong>Tremor, n (%)</strong></td>
<td>18 (60.0)</td>
<td>9 (30.0)</td>
<td>16 (53.3)</td>
<td><strong>0.048</strong></td>
</tr>
<tr>
<td><strong>Rigidity, n (%)</strong></td>
<td>11 (36.7)</td>
<td>7 (23.3)</td>
<td>13 (43.3)</td>
<td>0.242</td>
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<tr>
<td><strong>Bradykinesia, n (%)</strong></td>
<td>8 (26.7)</td>
<td>19 (63.3)</td>
<td>18 (60.0)</td>
<td><strong>0.006</strong></td>
</tr>
<tr>
<td><strong>Symmetry of signs, n (%)</strong></td>
<td>12 (40.0)</td>
<td>9 (30.0)</td>
<td>17 (56.7)</td>
<td>0.109</td>
</tr>
<tr>
<td><strong>RBD, n (%)</strong></td>
<td>7 (23.3)</td>
<td>1 (3.3)</td>
<td>7 (23.3)</td>
<td><strong>0.014</strong></td>
</tr>
<tr>
<td><strong>Hyposmia or anosmia, n (%)</strong></td>
<td>8 (20.0)</td>
<td>3 (10.0)</td>
<td>6 (20.0)</td>
<td>0.436</td>
</tr>
<tr>
<td><strong>Constipation, n (%)</strong></td>
<td>7 (23.3)</td>
<td>5 (16.7)</td>
<td>15 (50.0)</td>
<td><strong>0.019</strong></td>
</tr>
<tr>
<td><strong>Cognitive decline, n (%)</strong></td>
<td>14 (46.7)</td>
<td>16 (53.3)</td>
<td>12 (40.0)</td>
<td>0.584</td>
</tr>
<tr>
<td><strong>Depression, n (%)</strong></td>
<td>12 (40.0)</td>
<td>8 (26.7)</td>
<td>6 (20.0)</td>
<td>0.245</td>
</tr>
</tbody>
</table>

Between the three groups, frequencies of tremor (p=0.048), bradykinesia (p=0.006), constipation (p=0.019), and RBD (p=0.014) were statistically different (Table). Tremor was more frequent in normal group than in aPD group (p=0.05). Bradykinesia was more frequently observed in aPD group (p=0.011) or PD group (p=0.023) than in normal group. Constipation was statistically frequent in PD group than in aPD group (p=0.016). The frequency of RBD was higher in normal group or PD group than in aPD group with no significant difference (p=0.061).

**Conclusions:** Significant differences between the groups may suggest potential clinical features of patients with parkinsonism on predicting the nigrostriatal dopaminergic status.
ON-DEMAND SYMPOSIUM: PD, LBD DIAGNOSIS & CLINICAL TRIALS & ADVANCES IN TRANSLATIONAL DRUG DEVELOPMENT

SPOKEN LANGUAGE IMPAIRMENTS IN LEWY BODY TYPE DEMENTIAS: RESULTS OF A SCOPING REVIEW

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Aims: While awareness has grown regarding the debilitating language impairments arising in Primary Progressive Aphasia (PPA), progressive language difficulties are not unique to PPA, occurring across a range of dementias. In Lewy body dementia (LBD) types, a variety of spoken language difficulties can occur. The aim of this study was to review and identify the range, severity, and impact of spoken language difficulties in these forms of dementia, and to critique the research evidence on current language interventions.

Methods: A scoping review was conducted, searching the following databases: PubMed, MEDLINE, OVID-EMBASE, PsycINFO and SpeechBITE. Dementia types included Parkinson’s disease dementia (PDD), dementia with Lewy bodies (DLB), Progressive supranuclear palsy (PSP), and Cortico-basal syndrome (CBS). The QualSyst tool and the PEDro-P scale were used to assess methodological rigor.

Results: The search revealed 59 eligible studies in LBD (PDD=16, DLB=8, CBS=18, PSP=17), of which 85% (87/59) were rated as good quality according to QualSyst criteria. A range of expressive and receptive language impairments were identified, with full aphasic syndromes (non-fluent agrammatic PPA) described in people with CBS and PSP. No information was provided regarding severity of the language impairment in PDD and DLB, with 1 paper reporting on this within PSP, and 3 papers in CBS. Surprisingly, no papers described the impact of these language impairments on everyday living or presented data regarding language therapies to treat them.

Conclusions: Studies assessing the impact of spoken language impairments on everyday life in people living with LBDs are needed, together with trials of language therapy to support evidence-based treatment for these people.
LONGITUDINAL MEASUREMENTS OF DOPAMINE TRANSPORTER WITH [18F]FE-PE2I PET IN SUBJECTS WITH NON-ADVANCED PARKINSON'S DISEASE

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Aims: Dopamine transporter (DAT) PET with [¹⁸F]FE-PE2I has good reliability and repeatability in non-advanced Parkinson's disease (PD) (doi: 10.1186/s13550-020-00676-4). The aim of this study was to assess the suitability of [¹⁸F]FE-PE2I to measure longitudinal DAT changes in PD.

Methods: Forty patients with idiopathic Parkinson's disease (PD), Hoehn and Yahr stage below 3, were included in a longitudinal PET study with [¹⁸F]FE-PE2I. DAT availability (BPND) in caudate, putamen, substantia nigra, and sensorimotor striatum, was estimated with parametric imaging using Logan graphical analysis and cerebellum as reference region.

Results: Baseline and longitudinal PET data (interval: 2.14±0.14 years) were available for 17 patients in this ongoing study. Twelve were males; median age: 67 (range 46—73); symptom duration: 3 years (0.25—14); Hoehn and Yahr stage: 1 (1—2); mean LEDD: 396.2±239.2 mg. Mean baseline BPND, percentage DAT decline, and effect size (Cohen’s dz) were: caudate (1.84, -14.8%, 0.75), putamen (1.33, -8.8%, 0.66), substantia nigra (0.687, +3.3%, 0.008), sensorimotor striatum (0.827, -8.9%, 0.59).

Conclusions: Longitudinal [¹⁸F]FE-PE2I PET measurements in non-advanced PD demonstrate a decline of striatal DAT consistent with previous SPECT and PET studies. No obvious changes of DAT-availability were observed in the substantia nigra, which might indicate slower progression or compensatory changes. The effect sizes were numerically larger than those calculated from published [¹²³I]FP-CIT SPECT data (https://doi.org/10.1002/mds.27361), suggesting that [¹⁸F]FE-PE2I can be used as progression marker in clinical trials.
TARGETED URINE PROTEOME IDENTIFIES A DISTINCT BIOMARKER PANEL IN EARLY PARKINSON’S DISEASE

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1University Medical Center Göttingen, Department Of Neurology, Göttingen, Germany, 2University College London, Institute Of Child Health And Great Ormond Street Hospital, London, United Kingdom, 3University College London, Queen Square Institute Of Neurology, London, United Kingdom, 4Paracelsus-Elena Hospital, Center Of Parkinsonism And Movement Disorders, Kassel, Germany, 5University Medical Center Goettingen, Department Of Neurosurgery, Göttingen, Germany, 6Philips University, Department Of Neurology, Marburg, Germany

Aims: Peripheral pathology early in Parkinson’s disease (PD) enables the identification of biomarkers in peripheral biological fluids. Urine samples are easy to assess and show stable proteolytic dynamics, but less is known about urinary protein profiles in PD. We therefore aimed to identify protein biomarkers in urine of patients with PD.

Methods: For discovery, unbiased nano 2D-LC-TOF MS and a systematic literature search identified a panel of 696 proteins in PD and healthy controls (HC). For validation a targeted mass spectrometry assay (UPLC LC-MSMS) was developed measuring 59 proteins in (early morning) urine samples from baseline of newly diagnosed, unmedicated PD patients and matched HC from the observational, single center De Novo Parkinson (DeNoPa) cohort. Deep clinical phenotyping in DeNoPa continued with longitudinal follow-up of up to 10 years.

Results: Urine Samples from 81 PD (49 male, 60,5 %, mean age 64 years) and 48 HC (26 male, 54,2 %, mean age 64 years) subjects were analyzed. A total of 24 urine proteins expressed significantly different between PD and HC among these markers of neuroinflammation, alpha-synuclein metabolism, neuroprotection and autophagy. Statistical analysis showed that a cross validated PLS-DA classification model discriminates PD from HC with an AUC of 0.90.

Conclusions: This targeted MS analysis detected various markers distinguishing between PD and HC in easily accessible urine samples, which needs further validation including prodromal subjects. This targeted urine test could thereby lead to a biomarker screen for large population-based cohort studies to identify subjects at risk to develop PD.
PLASMA NEUROFILAMENT LIGHT IS MORE STRONGLY CORRELATED TO COGNITIVE DECLINE IN HIPPOCAMPAL-SPARING AD SUBTYPE THAN IN OTHER AD SUBTYPES

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Karolinska Institutet, Division Of Clinical Geriatrics, Department Of Neurobiology, Care Sciences And Society, Stockholm, Sweden

Aims: Recent studies suggest plasma neurofilament light (NFL) to be valuable biomarker for faster cognitive decline in patients with Alzheimer's Disease (AD) dementia. Recently, AD subtyping based on MRI or PET have been used to classify patients into AD phenotypes with possible different cognitive prognosis. Our aim was to investigate the interaction between plasma NFL and different AD subtypes to predict cognitive decline.

Methods: From the ADNI dataset were included AD subjects (Aβ PET+) with at least a plasma NFL measurement and an MRI scan for subtyping AD subjects following the Murray-Dickson algorithm implemented by Risacher et al. (2017). In total, 134 AD (Aβ+) subjects were selected and subtyped in Limbic predominant (Lp) (18.7%), typical AD (67.2%) and Hippocampal-sparing (HpSp) (14.2%) subtypes. ADNI_MEM, a composite score, was used as proxy for cognition. Linear mixed models were tested to predict cognition decline up to 4 years of follow up (22±11 months, mean±SD). The contribution of age, gender, education, and baseline cognition was tested (AIC/BIC), age and baseline cognition were included in the final model.

Results: The AD subtypes did not differ in age (73±8 ys), cognition (-0.8±0.7), nor plasma NFL (log) (3.8±0.4) but differed in gender. The interaction between time, plasma NFL (log) and subtype HpSp was significant in predicting the cognitive decline, differently from the other subtypes (p < 0.02).

Conclusions: Plasma NFL levels in AD patients with the Hippocampal-sparing subtype appeared to show faster cognitive decline than patients with other AD subtypes, suggesting a possible prognostic value of plasma NFL in this subtype.
ON-DEMAND SYMPOSIUM: AD IMAGING, BIOMARKERS, DIAGNOSTICS, CLINICAL TRIAL DESIGNS

RETINAL PATHOLOGICAL FEATURES AND NONINVASIVE RETINAL IMAGING IN PRODROMAL AND CLINICAL ALZHEIMER’S DISEASE

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Aims: Explore topographical distribution of retinal pathology in Alzheimer’s disease (AD) patients, assess relations with disease status, and investigate potential utility of noninvasive retinal amyloid and vascular imaging as tools to predict cognitive decline.

Methods: Spatial distribution of amyloid β-protein (Aβ₄₂), hyperphosphorylated (p)Tau, vasculopathy, gliosis, atrophy, and intraneuronal Aβ oligomers were determined in postmortem retinas of mild cognitively impaired (MCI) and AD patients versus normal cognition controls. Proteome signatures of AD retinas and brains were analyzed by mass spectrometry. Retinal curcumin-fluorescence imaging (RFI) studies were conducted to establish whether retinal amyloid and/or vascular features correlate with brain atrophy and neurocognitive status.

Results: Prominent retinopathy is found in MCI and AD patients, affecting inner layers and peripheral subregions early, and propagating to central outer segments in advanced disease. Retinal Aβ₄₂, oligomers, pTau, macrogliosis and Aβ-phagocytosing microglia, and atrophy correlated with cerebral ATN and cognitive scores. Distinct proteomic profiles of AD retinas were identified, displaying greatest overlap with frontal cortices. Proteome analysis of AD retinas suggests upregulated inflammation and neurodegeneration and downregulated mitochondrial- and photoreceptor-related pathways. In clinical trials, cognitive scores separated retinal RAI and vessel tortuosity index (VTI). Combined proximal mid-periphery amyloid count and venous VTI index exhibited significant differences between cognitively impaired and cognitively normal subjects and correlated with both verbal memory and cognitive-related quality of life scores.

Conclusions: Our histological and biochemical evidence identify and map AD retinopathy with tight relationship to brain pathology and cognition. Further, proximal mid-periphery amyloid count predicted hippocampal atrophy, and combined with retinal venular VTI correlated with cognition.
PREDICTING ACCUMULATION OF TAU PLAQUES IN CEREBRAL CORTEX WITH MULTIVARIATE MRI MORPHOMETRY MEASUREMENTS

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Aims: One of the hallmarks of Alzheimer’s disease is the accumulation of tau plaques in human brains. However, detecting tau pathology is either invasive or quite costly and not widely available. In our previous work, structural MRI-based hippocampal multivariate morphometry statistics (MMS) showed superior performance as effective neurodegenerative biomarkers for preclinical AD and Patch Analysis-based Surface Correntropy-induced Sparse coding and max-pooling (PASCS-MP) has excellent ability to generate low-dimensional representations with strong statistical power for brain amyloid prediction. In this work, we apply these to predict Tau deposition in Braak12 and Braak34 brain regions.

Methods: In Fig.1, MMS are first extracted from MR images. Panel 2 shows our PASCS-MP method. We randomly select patches of MMS on the hippocampal surface. Then, Sparse-coding and max-pooling are used to generate representations for these subjects. Finally, ridge regression models are trained with these representations to predict Braak12/Braak34 measurements.
Results: We evaluate our framework on 925 subjects from ADNI. The Braak12 and Braak34 predicted by the representations from our MMS and PASCS-MP are closer to the real values compared to the measures derived from other approaches such as hippocampal surface area and volume, and shape morphometry features based on spherical harmonics (SPHARM), as the RMSE shown in Fig.2 and Pearson correlation in Fig.3.
Conclusions: MMS-based representations refined by PASCS-MP achieve superior performance in predicting the measurements of Tau deposition. In the future, we will use this framework to study other AD-related regions of interest and further improve the framework to visualize the disease-related features on the surface.
ON-DEMAND SYMPOSIUM: AD IMAGING, BIOMARKERS, DIAGNOSTICS, CLINICAL TRIAL DESIGNS

DEVELOPMENT OF A DISEASE PROGRESSION MODEL TO PREDICT LONGITUDINAL TRAJECTORY OF ALZHEIMER’S DISEASE ASSESSMENT SCALE–COGNITIVE SUBSCALE (ADAS-COG11) IN MILD TO MODERATE AD

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Aims: We developed a disease progression model for characterizing longitudinal progression of ADAS-Cog11 scores in mild to moderate Alzheimer’s disease (AD) to serve as a reference for natural disease progression and help inform the assessment of potential treatment effect for Lauriet trial [NCT03828747; semorinemab]).

Methods: We used nonlinear mixed-effect population modeling to describe changes over time in ADAS-Cog11 scores in mild to moderate AD participants (baseline MMSE 16-21) from the Alzheimer’s Disease Neuroimaging Initiative (ADNI) (n=54) and placebo arms from several Roche/Genentech trials (n=155). The model structure and model building process were as previously described¹.

Results: Our disease progression model for ADAS-Cog11 in the mild to moderate AD was developed to predict the placebo progression for Lauriet trial. The model was successfully validated (via visual predictive checks) by assessing its ability to predict ADAS-Cog11 progression in model building dataset (n=209) and in the mild-moderate patients in the CREAD and Tauriel trials which were not used for model development (n=164). The model enabled us to predict the change in ADAS-Cog11 in absence of active treatment for each patient for comparison with on-treatment observed values in the same patients to aid in assessing a treatment effect.

Conclusions: We developed a disease progression model for characterizing longitudinal natural progression of ADAS-Cog11 scores in mild to moderate AD. Results support the utility of this model to aid in assessing treatment outcomes in future AD clinical trials by predicting hypothetical off-treatment ADAS-Cog11 scores for patients in the active arm. ¹ Jamalian, S. et al. CTAD 2020
Aims: Understanding the mechanism of Alzheimer’s disease (AD) has always been challenging. AD progression is more closely related to tau as opposed to amyloid deposition over time. We designed a novel deep learning approach to investigate if transcriptome changes can predict CSF-tau levels. To our best knowledge, this is the first study to use deep learning for cerebrospinal fluid (CSF)-tau prediction from transcriptomic data in the prodromal AD stages.

Methods: 384 subjects (CN=118, MCI=266) were selected from ADNI cohort. We used linear regression to conduct data-driven dimensionality reduction of the transcriptomic vector by selecting the transcripts that have significant associations with hippocampal volume (p<0.05). K-means clustering and principal component analysis were employed to further select representative genes. Then we designed a deep neural network model to learn the hierarchical features in the selected gene vectors to predict CSF-tau abnormality.

Results: 2,364 from 49,386 transcripts were selected after the two-step dimensional reduction. Using these selected transcripts, as well as age and gender, our deep neural network achieved an accuracy of 67% and an area under the curve (AUC) of 68% in predicting CSF total tau levels. We also evaluated CSF phospho-tau prediction, but the accuracy was only 53%.

Conclusions: We designed a novel deep learning model to investigate the associations between human transcriptome and CSF total tau and phospho-tau changes in prodromal AD and found better predictive accuracy for CSF total tau – a neurodegenerative marker. Our model has shown attractive potential to improve our understanding of the genetic drivers of neurodegeneration in AD.
Aims: Transitional cognitive decline (TCD) in the Alzheimer’s continuum in 2018 NIA-AA Research Framework may be a better time window than mild cognitive impairment (MCI) for early intervention of subjects with Alzheimer disease (AD) risks. TCD may involve memory and other cognitive domains which subjective cognitive decline (SCD) tests cannot assess. Additionally, SCD tests are better to be corroborated by objective cognitive testing. Here, a rapid performance-based screening instrument (QuickCog, 10.13140/RG.2.2.25923.12327/1) for detecting TCD was developed.

Methods: The QuickCog, SCD-9, and MoCA tests were used to examine 88 college students (22.8 ± 2.9 years, 18–29 years, 40 males) and 60 community-dwelling elderly people living independently (69 ± 5.3 years, 60–82 years, 18 males) with normal instrumental activities of daily living scores.

Results: Compared to the young students, the elderly presented greater decline in QuickCog scores than MoCA and SCD-9 (young vs. elderly: QuickCog, 25.22 ± 2.42 vs. 18.05 ± 3.44; MoCA, 27.48 ± 2.03 vs. 22.28 ± 3.36; SCD-9, 3.72 ± 2.21 vs. 4.58 ± 1.72).

Conclusions: The QuickCog test may be used as a sensitive instrument to assess TCD. The test is designed to avoid ceiling effects when assessing individuals presenting “normal” performance with objective cognitive test such as MoCA and a SCD test such as SCD-9. The QuickCog test emphasizes a constant pace during testing to build a good baseline across subjects. The completing duration of several items of the test provides an additional dimension than scores.
PLASMA BIOMARKERS FOR CLASSIFICATION OF AD PATHOLOGY BY A FULLY AUTOMATED IMMUNOASSAY SYSTEM (HISCL SERIES)

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Aims: ATN classification system (A: β-amyloid (Aβ) deposition, T: pathogenic tau, N: neurodegeneration) has been widely accepted in characterization of Alzheimer’s disease (AD) based on its pathological process. Evaluation of ATN biomarkers requires cerebrospinal fluid (CSF) sampling and/or neuroimaging. To accelerate their practical diagnostic application, it is desirable to establish a simple diagnostic method such as a blood-based test. Recently, we have developed plasma Aβ₁₋₄₀, Aβ₁₋₄₂, threonine-181 phosphorylated tau (p-tau181), and total-tau immunoassays and reported that the levels of these biomarkers were significantly different between AD and cognitively normal (CN) groups. Here, in addition to above biomarkers, we present the performance of newly developed neurofilament light chain (NfL) assay as a candidate for “N” category by measuring plasma and CSF samples.

Methods: We measured Aβ₁₋₄₀, Aβ₁₋₄₂, p-tau181, total-tau and NfL in commercially available plasma and CSF samples on a fully automated immunoassay platform (HISCL™ series). The concentration of plasma and CSF biomarkers were determined to evaluate the differences between AD, MCI, and CN groups.

Results: Plasma Aβ₁₋₄₂/Aβ₁₋₄₀ ratio was lower, p-tau181 and total-tau levels were higher in the AD group compared to MCI and/or CN groups. Plasma and CSF mean levels of NfL were higher in AD compared to MCI and/or CN groups, suggesting that plasma NfL level correlated with AD pathological stages as well as Aβ, p-tau181, and total-tau.

Conclusions: Plasma biomarkers showed characteristic distribution in AD, MCI, and CN groups on a HISCL™ platform. Our results indicated that NfL assay may have sufficient performance as a candidate of “N” category.
FULLY AUTOMATED PLASMA BETA-AMYLOID IMMUNOASSAYS PREDICT AMYLOID PATHOLOGY DEFINED BY AMYLOID PET

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Aims: Alzheimer’s disease (AD) is pathologically characterized by the accumulation of β-amylloid protein (Aβ). Several clinical trials for disease modifying therapies (DMTs) targeting Aβ have been conducted, and recently the U.S. Food and Drug Administration approved the first DMT, aducanumab for the treatment of AD. Thus, the necessity of rapid and cost-effective diagnostic method for detecting the amyloid pathology is increased. In a previous study, we reported the performance of our plasma Aβ assay to predict amyloid pathology, and recently, we have updated our assays for clinical usage. Here, we present the clinical performance of our updated assay to predict amyloid pathology.

Methods: Plasma Aβ₁-40 (Aβ40) and Aβ₁-42 (Aβ42) were quantified using a fully automated platform (HISCL™ series), which requires only 30 μL of plasma per assay and 17 min to complete. We used plasma samples from subjects with mild cognitive impairment due to AD and mild AD. Amyloid positivity of all subjects was determined by amyloid PET using florbetaben, florbetapir, or flutemetamol. The concentration ratios of Aβ42 and Aβ40 (Aβ42/Aβ40) were calculated and compared to amyloid PET results.

Results: Aβ42/Aβ40 was decreased in amyloid positive group compared with negative group, suggesting that our assay may reflect amyloid pathology. The AUROC was improved significantly from our previous results.

Conclusions: We have developed the plasma Aβ assay to predict amyloid positivity determined by PET. Our assay employed a fully automated easy-to-use platform, indicating that our method may easily adapt to routine clinical practice, and contribute to the treatment of AD.
89Zr-immuno-PET as a translational tool to quantify brain uptake and biodistribution of monoclonal antibodies.

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Aims: Demonstrating clinical efficacy of monoclonal antibodies (mAbs) acting in the brain is challenging, which is exemplified by the recent FDA approved Aduhelm (aducanumab). Molecular imaging advances drug development, by facilitating the visualization and quantification of therapeutic effects of mAbs in the brain. An aducanumab derivative, Abeta-mAb, and a bispecific variant having a transferrin targeting moiety for enhanced brain uptake, Abeta-mAb-scFab8D3, were labelled with 89Zr and their brain distribution determined with Positron Emission Tomography (PET) in APP/PS1 transgenic Alzheimer mice and compared to the brain biodistribution of [11C]PIB.

Methods: APP/PS1 mice (10 months) were injected with 1 mg/kg 89Zr-radiolabeled mAb and imaged at day 1, 3 and 7 with PET. Ex vivo biodistribution and immunofluorescence staining of brain tissue were performed on day 7 post-injection. Additionally, brain distribution of [89Zr]Zr-DFO*-Abeta-mAb-scFab8D3 was compared to amyloid-beta imaging using [11C]PIB with PET for different plaque loads in 3, 7 and 10 month old APP/PS1 mice. For all experiments WT mice served as control.

Results: A 7-fold higher brain uptake was observed for [89Zr]Zr-DFO*-Abeta-mAb-scFab8D3 compared to [88Zr]Zr-DFO*-Abeta-mAb at day 7. Immunofluorescence staining demonstrated co-localization of the mAb with the amyloid-beta plaques. In contrast to [11C]PIB, [89Zr]Zr-Abeta-mAb-scFab8D3 showed a specific amyloid-beta signal in APP/PS1 compared to WT mice already at 3 months old, whilst [11C]PIB was just able to detect a specific signal at 10 months.

Conclusions: This study demonstrates that 89Zr-Immuno-PET, using DFO* as chelator, enables visualization and quantification of brain distribution of mAbs and provides the means for exploring the
Figure 1: Brain uptake day 7 p.i. of $^{89}$Zr-DFO*-labeled monospecific Abeta-mAb and the bispecific Abeta-mAb-scFab8D3 in APP/PS1 transgenic (TG) and WT control mice. Top: PET imaging; one representative PET/CT image shown per group. Bottom: Ex vivo brain biodistribution expressed as %ID/g (mean ±SD, n = 5 animals per group). Significant differences between the groups are marked with asterisks (***p < 0.001; ****p < 0.0001).
AMYLOID PATHOLOGY IS ASSOCIATED WITH REGION-SPECIFIC METABOLIC DECLINE IN THE PREFRONTAL CORTEX OF PATIENTS WITH ATTENTION-DEFICIT/HYPERACTIVITY DISORDER AND MCI

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1Hospital de Clínicas de Porto Alegre, Adhd Outpatient Program & Development Psychiatry Program, Porto Alegre, Brazil, 2University of Pittsburgh, Department Of Psychiatry, Pittsburgh, United States of America, 3Universidade Federal do Rio Grande do Sul (UFRGS), Department Of Biochemistry, Porto Alegre, Brazil, 4Sahlgrenska Academy at the University of Gothenburg, Department Of Psychiatry And Neurochemistry, Göteborg, Sweden, 5McGill University, Mcgill University Research Center For Studies In Aging, Montreal, Canada

Aims: Patients with attention-deficit/hyperactivity disorder (ADHD) have an increased risk of developing MCI and AD. No study has evaluated whether amyloid pathology is associated with specific signatures of brain metabolic decline in patients with concomitant MCI and ADHD (ADHD-MCI).

Methods: We performed a preliminary case-control study nested within ADNI. We identified 17 patients with ADHD-MCI and compared them to 51 MCI (MCI-control) and 51 cognitively unimpaired (CU) individuals paired for age (mean=71y), sex (51% female), APOE4, psychiatric comorbidities, and years of study.

Results: Cognitive function was similarly decreased in both MCI-control and ADHD-MCI compared to CU (figure 1). CSF levels of AD pathology were significantly altered in MCI-control (decreased Aβ42, increased total tau, and p-tau) compared to both CU and ADHD-MCI, and no difference between ADHD-MCI and CU was observed (figure 2). Correlation analyses, however, demonstrated that decreased levels of CSF Aβ42 were associated with impaired cognitive function in both MCI-control and ADHD-MCI, but not in CU. Mediation analyses indicated that in MCI-control the effects of Aβ42 in cognition were mediated by decreased glucose metabolism (assessed with FDG-PET) in temporoparietal regions. In contrast, in ADHD-MCI, the effects of Aβ42 in cognition were mediated by decreased glucose metabolism in prefrontal regions (figure 3).
Figure 1

Cognition

mPACC score

CU  MCI-control  ADHD-MCI

p=.001

p=.001
Conclusions: Findings indicate that lower levels of AD pathology are sufficient to induce cognitive impairments in patients with ADHD, suggesting decreased cognitive reserve and/or decreased resilience.
in this population. Aβ deposition was associated with specific signatures of brain glucose hypometabolism in the prefrontal cortex of ADHD-MCI.
Aims: The goal of this study was to test the ability of a novel, electronic version of the CDR (eCDR), developed by an automatic scoring algorithm, to detect mild cognitive impairment in an online setting.

Methods: Data were collected through the Brain Health Registry (BHR) from older adults diagnosed as cognitively unimpaired (CU; n=113) or mild cognitive impairment (MCI; n=31). Logistic regression was employed to assess how the eCDR performs for the prediction of MCI using 5 models containing the: i. eCDR global score; ii. eCDR domain scores; iii. eCDR item response theory (IRT) domain scores; iv. full model; v. stepwise regression.

Results: The eCDR global score with covariates detected diagnosis with 81% accuracy and the eCDR box scores with 83% accuracy. The optimal collection of items for predicting MCI were extracted using backwards stepwise logistic regression, selecting family history, race, self-reported memory concern, the orientation domain box score, and IRT scores from the memory domain, personal care domain and global score. Model 5 achieved the highest performance in all measures of detection, except specificity.

Conclusions: The eCDR is an unsupervised instrument with promising utility to identify older adults within the prodromal stage of AD remotely. Future studies will explore the relationship between the eCDR and the CDR and validate the eCDR against AD biomarkers.
ON-DEMAND SYMPOSIUM: AD IMAGING, BIOMARKERS, DIAGNOSTICS, CLINICAL TRIAL DESIGNS

SEX DIFFERENCES IN BIOMARKERS AND COGNITIVE PROFILE IN EARLY-ONSET ALZHEIMER’S DISEASE

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1Hospital Clínic of Barcelona-IDIBAPS. Universitat de Barcelona, Barcelona, Alzheimer’s Disease And Other Cognitive Disorders Unit, Neurology Service., Barcelona, Spain, 2Institut Hospital del Mar d'Investigacions Mèdiques, Unitat De Deteriorament Cognitiu I Transtorns Del Moviment, Neurology Service, Barcelona, Spain, 3University of Barcelona, Institute Of Neurosciences. Department Of Biomedicine, Faculty Of Medicine, Barcelona, Spain, 4Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas, (ciberned), Barcelona, Spain, 5Global Brain Heath Institute, Atlantic Fellow For Equity In Brain Health, Dublin, Ireland, 6Global Brain Heath Institute, Atlantic Fellow For Equity In Brain Health, San Francisco, United States of America, 7Hospital Clinic of Barcelona-IDIBAPS, Image Diagnostic Centre, Barcelona, Spain, 8Biomedical Research Networking Center in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Biomedical Imaging Group, Barcelona, Spain

Aims: To study sex differences in CSF biomarkers, brain MRI and cognition in early-onset Alzheimer’s disease (EOAD).

Methods: We included 106 subjects: 62 EOAD (A+T+N+, MMSE>15) and 44 healthy controls (HC; A-T-N-). They underwent lumbar puncture, 3T-MRI scan, neuropsychological assessment [EOAD: 54; HC: 43] and APOE genotyping. We also analyzed CSF Neurofilament light chain (NF-L) levels [EOAD: 54; HC: 40]. We used Freesurfer atlas-defined parcellations to measure cortical thickness (CTh) and subcortical volumes of MRI, including hemispheric measures. Volume was adjusted by intracranial volume. Cognition was z-normalized using HC. Adjusted lineal models were used to analyze differences between sexes across groups.

Results: There were no demographic differences across groups. Significant differences in MMSE were observed. APOE ε4 carriers frequency was higher in female-EOAD/male-EOAD than female-HC (p<0.05; Table1). No sex differences in cognition were found across groups. Differences in hemispheric CTh between EOAD and HC were driven by the female group. In EOAD, differences in left parahippocampal CTh were found between sexes (corrected p<0.05, Figure1). Higher P-Tau and T-Tau levels were found in female-EOAD (P-Tau=136.6 pg/mL (55.6), T-Tau=1021.2 pg/mL (416.1)) compared to male-EOAD (P-Tau= 97.9(28.3); T-Tau=692.5(318)). Female-HC exhibited lower NF-L levels than male-HC (422.1(136.1)/553.2(161.3), respectively) (all p<0.05, Bonferroni corrected).
<table>
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<th>EOAD=62</th>
<th>HC=44</th>
<th>p value</th>
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<tr>
<td>Age at onset (years)</td>
<td>62 56.9(4.1)</td>
<td>N/A N/A</td>
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<tr>
<td>MMSE</td>
<td>59 23.1(3.8)</td>
<td>44 28.9(1.5)</td>
<td>6.3(^{17})</td>
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<td>Age at MRI (years)</td>
<td>62 60.1(4.2)</td>
<td>44 58.3(4.9)</td>
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<td>Age at LP (years)</td>
<td>62 60.1(4.5)</td>
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<td>Age at cognitive assessment (years)</td>
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<td>Education (years)</td>
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<td>44 11.7(3.6)</td>
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<tr>
<td>Age at onset (years)</td>
<td>34 57.5(4.3)</td>
<td>28 56.3(3.8)</td>
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<tr>
<td>MMSE</td>
<td>31 22.4(4)(^{a,b})</td>
<td>28 23.9(3.3)(^{a,b})</td>
<td>32 28.7(1.7)(^{c,d})</td>
<td>12 29(1)(^{c,d})</td>
</tr>
<tr>
<td>Age at MRI (years)</td>
<td>34 60.6(4.1)</td>
<td>28 59.4(4.3)</td>
<td>32 58.6(4.5)</td>
<td>12 57.8(6)</td>
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<tr>
<td>Age at LP (years)</td>
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<td>28 59.3(4.3)</td>
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<td>12 57.7(6)</td>
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<td>Age at cognitive assessment (years)</td>
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<td>28 12.5(3.2)</td>
<td>32 11.3(3.5)</td>
<td>12 12.8(3.7)</td>
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<td>19(^a)</td>
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<td>4(^c,d)</td>
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</table>

Table 1. Demographic and clinical characteristics. EOAD and HC were compared using t Student tests for continuous variables and \(\chi^2\) tests for the sex and APOE. ANOVA with post hoc analysis and Fisher’s test were used to analyze differences across sex groups. Key: a. Significantly different from HC-female; b. Significantly different from HC-male; c. Significantly different from EOAD-female; d. Significantly different from EOAD-male; HC healthy control; SD; standard deviation; MRI, magnetic resonance imaging; LP, lumbar puncture.
Conclusions: In EOAD, the pattern of brain atrophy was similar between sexes. However, females showed lower hemispheric CTh and more marked differences from HC. No differences were found in cognition. Females showed higher Tau levels in EOAD and lower NfL levels in HC. Further studies are necessary to elucidate the existence of sex differences in EOAD.

Figure 1. MRI significant differences in regional cortical thickness and subcortical gray matter volume between groups and sexes. We show standardized β coefficients p<0.05 (Bonferroni corrected) from linear models used to explore between-group differences in MRI measures, using age and APOE as covariates. MMSE was also used as covariate when analyzing differences between sex in EOAD. Key: EOAD early-onset AD, HC healthy control, Std. β, standardized β coefficients.
ON-DEMAND SYMPOSIUM: AD IMAGING, BIOMARKERS, DIAGNOSTICS, CLINICAL TRIAL DESIGNS

INFLAMMATORY PATHWAYS ASSOCIATED WITH TAU PATHOLOGY IN ALZHEIMER'S DISEASE

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Aims: To study the association of inflammation composite indexes with tau pathology across the aging and AD spectrum. We hypothesize that newly proposed inflammatory indexes representing different pathways in the inflammatory cascade, rather than individual proteins, better reflect tau propagation in AD.

Methods: A panel of 96 inflammation-related markers was measured in the CSF of 35 cognitively unimpaired(CU) and 25 cognitively impaired(CI) individuals from the McGill TRIAD cohort. These 96 proteins were clustered by ontogenetic similarity and physical interaction using STRING. A z-scored composite representing each cluster was created for each individual. Plasma p-Tau181 was measured using Simoa, and CSF YKL-40 and GFAP by ELISA.

Results: Of seven clusters identified in our protein network analysis, four positively correlated with plasma p-Tau(Fig.1). These four clusters represented specific processes previously linked with AD, such as tumor necrosis factor, leukocyte response to cytokine stimulus, glial cell-related neurotrophic factor and JAK-STAT signaling. Linear regressions demonstrated that these clusters did not discriminate CU and CI but significantly differed between tau positive and negative(Fig.2). Regressions suggested that the newly identified clusters were more strongly related to plasma p-tau positivity than traditional markers of glial activation.
Conclusions: Our data-driven cluster approach supported that inflammation is closer related to elevated p-tau proteinopathy than with the AD diagnosis. Our results demonstrated, for the first time, that a composite of inflammatory proteins with ontogenetic similarities better identify tau pathology than single markers. Our results suggest that therapeutic approaches targeting inflammatory pathways, rather than single proteins, may be an alternative to halt tau propagation in AD.
EXOSOMES ENTER GLIAL CELLS THROUGH AN ACTIN-NETWORK ENDOCYTIC PATHWAY MEDIATING A-SYN TRANSMISSION

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Aims: Exosomes have recently emerged as key players in cellular communication in both physiological and pathological processes in the brain, particularly in synucleinopathies. However, the interplay between exosomes and the different cell types of the brain (neurons, astrocytes, microglia) has yet to be elucidated. The current study aims to examine the intracellular trafficking pathway of exosomes in primary neuronal and glial cells linked with the exosome-associated α-syn transmission.

Methods: Mouse cortical neurons, microglia and astrocytic primary cultures were incubated with DiI-stained mouse brain-derived exosomes, in the absence or presence of recombinant fibrillar human α-Syn. The internalization and trafficking pathways were analysed by immunofluorescence in cells treated with pharmacological reagents that block the major endocytic pathways.

Results: Brain-derived exosomes were internalized by both microglia and astrocytes but less efficiently by cortical neurons. Colocalization of exosomes with early and late endocytic markers (Rab5, Lamp1) indicated that exosomes are sorted to endolysosomes. Treatment with Cytochalasin b, that blocks actin-dependent phagocytosis and macropinocytosis, inhibited exosome entry into glial cells. Dynasore, that inhibits dynamin-dependent endocytosis, did not appear to affect exosome uptake, however the sorting to late endosomes was delayed. Exosome-associated fibrillar α-Syn was efficiently uptaken and detected within glial cells.

Conclusions: Exosomes enter glial cells through an actin network-dependent phagocytic pathway and are sorted to endolysosomes for degradation or recycled back to the plasma membrane. Further, brain-derived exosomes are capable of mediating cell-to-glia transmission of pathological α-Syn that is also likely targeted to the endocytic pathway for clearance.
ON-DEMAND SYMPOSIUM: AMYLOIDS, TAU ALPHA-SYNUCLEIN, PRIONS. PROTEIN MISFOLDING; DISEASE MECHANISMS & DETECTION 1

STRUCTURAL CHARACTERIZATION OF THE α-SYNUCLEIN STRAINS AMPLIFIED FROM THE PARKINSON'S DISEASE AND MULTIPLE SYSTEM ATROPHY PATIENT CSF USING CRYO-ELECTRON MICROSCOPIC

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Aims: Misfolded α-synuclein (αSyn) adopts alternative conformations in different synucleinopathies and able to spread the abnormal structures in a prion-like manner through anatomically connected brain regions. Recently, we developed the protein misfolding cyclic amplification (PMCA) assay for high sensitive detection of αSyn aggregates in patients affected by Parkinson’s disease (PD) and related synucleinopathies. Interestingly, we observed different biochemical, biophysical and structural properties of αSyn aggregates amplified from patients affected by PD and a clinically similar synucleinopathy, termed multiple system atrophy (MSA). Here, we investigated the structural features of these amplified aggregates at high resolution which will be crucial for understanding the disease mechanisms and for the development of the therapeutic interventions.

Methods: To this end, we used cryo-electron-microscopy (Titan Krios) to solve the structures of the αSyn strains present in PD and MSA patients’ CSF.

Results: In the case of PD, we found four distinct structures of αSyn filaments. The structures, resolved at resolutions of 3.6 – 5.0 Å, showed different backbone arrangements of the αSyn subunits in the filament axis. On the contrary, MSA cases showed the presence of three distinct filament conformations. One of the filament conformations (3.8 Å) is strikingly similar to the αSyn structure present in the MSA brain. These results suggest that PMCA faithfully replicates the structure of αSyn aggregates.

Conclusions: Our study shows the high resolution structural difference of αSyn filaments present in PD and MSA patients. Our findings may aid in the design of diagnostic and therapeutic strategies for these diseases.
INVESTIGATING THE ROLE OF LYROSOMAL ACIDIFICATION IN ALPHA SYNUCLEIN-INDUCED PARKINSON’S DISEASE MODELS USING NOVEL ACIDIC NANOPARTICLES

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Aims: Parkinson disease (PD) is the second most common neurodegenerative disease in the world today. PD results mainly from the death of dopaminergic neurons in the substantia nigra (SNc) with accumulation of toxic alpha synuclein (α-syn) aggregates within Lewy bodies (LBs) that are unable to be degraded. Recent studies in both cellular and mouse models of α-syn induced PD have indicated that dysfunctions in the endolysosomal autophagy pathway contribute to α-syn accumulation and play a role in PD pathogenesis. While most studies have focused on promoting lysosomal enzyme activity to restore lysosome function and reduce α-syn pathology, direct activation of lysosomal acidification by lowering its pH has not been shown. In this study, we used a novel pH-activable acidic nanoparticles (acNPs) as an agent to induce lysosome acidification in cellular models of α-syn.

Methods: We have synthesized a polymeric based acidic nanoparticle (acNPs) that can be activated in dysfunctional lysosomal pH environment to release acid to further lower lysosomal pH.

Results: We have shown that the restoration of lysosomal acidification with acNPs led to a rescue of autophagic function in SH-SY5Y and N2a cells, thereby leading to decreased accumulation of α-syn in cells and restoration of cellular viability. We also showed that acNPs reduce α-syn secretion by measuring the content of α-syn in the cell culture media.

Conclusions: acNPs can serve as a tool to study the mechanism of lysosomal acidification in α-syn induced PD models and can serve as a potential therapeutic for PD.
Aims: Neuromelanin granules (NMGs) are organelle-like structures present in the human substantia nigra pars compacta. Besides neuromelanin, NMGs contain proteins, lipids and metals. As NMG-containing dopaminergic neurons are especially lost in Parkinson’s disease and dementia with Lewy bodies (DLB), NMGs may play a role in neurodegenerative processes. Until now, this role is not completely understood and needs further investigation. We therefore set up a proteomic study to identify differences in the proteomic profile of NMGs from DLB patients (n=5) compared to healthy controls (CTRL, n=5).

Methods: We used a laser microdissection and mass-spectrometry-based approach. Significantly differential proteins were validated by parallel reaction monitoring (PRM) experiments.

Results: Of 3,090 identified proteins, 81 proteins were found to be significantly differential between NMGs of DLB and CTRL. Among them, alpha-synuclein (p=0.001) and protein S100A9 (p=0.019) displayed a higher abundance in NMGs of DLB patients. As S100A9 is known to enhance the formation of toxic alpha-synuclein fibrils, this finding points towards an involvement of NMGs in pathogenesis. In addition, proteins related to stress granules (SGs) were higher abundant in NMGs of DLB patients, indicating a link between NMGs and SGs.

Conclusions: In general, our study shows clear differences in the NMG protein patterns of DLB and CTRL suggesting an involvement of NMGs in the neurodegenerative processes in DLB. Moreover, our data exhibit a yet undescribed link between NMGs and SGs, which either means that NMGs share functionalities of SGs or that SGs form in near proximity to NMGs. Future functional studies are needed to further explain this phenomenon.
Aims: α-Synuclein accumulation into dopaminergic neurons is a pathological hallmark of Parkinson’s disease. Fatty acids partially regulate α-synuclein accumulation, and fatty acid-binding protein 3 (FABP3) associates with the aggregations. FABP3 is rich in dopaminergic neurons and interacts with dopamine D2 receptors, specifically the long type (D2L), abundant in caveolae. Here, we investigated the impact of dopamine D2L receptors and FABP3 in the process of α-synuclein uptake and its aggregation.

Methods: We prepared mesencephalic neurons derived from dopamine D2L+/−, dopamine D2 receptor null (D2 null), FABP3−/−, and wild-type C57BL6 mice, and analyzed the uptake ability of ATTO fluorescence-conjugated α-synuclein monomers and fibrils.

Results: We found that D2L receptors are co-localized with FABP3. Immunocytochemistry revealed that tyrosine hydroxylase (TH)+ FABP3+/−, D2L−/− or D2 null neurons do not take up α-synuclein monomers. The deletion of α-synuclein C-terminus abolished the uptake to dopamine neurons. Likewise, dynasore, a dynamin inhibitor, and caveolin-1 knockdown also inhibited the uptake. Additionally, D2L and FABP3 were critical for α-synuclein fibrils uptake. D2L and accumulated α-synuclein fibrils were FABP3-dependently co-aggregated, which accelerated proteasomal degradation of TH protein. FABP3 was also essential for MPP+–induced reduction of mitochondrial activity along with the production of reactive oxygen species.

Conclusions: These data indicate that the FABP3 coupled with dopamine D2L receptors assisted by caveola structure is critical for α-synuclein uptake into dopaminergic neurons and the loss of neuronal homeostasis, suggesting a novel pathogenic mechanism of synucleinopathies, including Parkinson’s disease.
CONCENTRIC BETA BARREL MODELS OF ALPHA SYNUCLEIN, AMYLOID BETA, AND IAPP

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Aims: The major objective of analyzing amyloid structures is to develop effective ways to prevent, treat, and/or cure the devastating diseases they cause. Amyloid beta (Aβ) of Alzheimer’s disease, α-synuclein of Parkinson’s disease, and Amylin (IAPP) of Type 2 Diabetes form large fibrils. Evidence is increasing however that much smaller oligomers are more toxic and that these oligomers can form toxic transmembrane ion channels.

Methods: The quest to determine structures of these smaller assemblies can appear hopeless. Amyloid peptides are shapeshifters; they assume countless forms that are often present simultaneously and likely have partially occupied secondary structures. Numerous factors affect Aβ assemblies; e.g., ions and heavy cations, lipids, fatty acids, concentration, time, method of isolation and preparation, location in the body, initial seed conformation, length of the peptide, mutations, and oxidation. Also, they appear to interact with multiple receptors, and with other amyloids. A potential saving grace is that symmetric β-barrels often have well defined structures.

Results: Our models of numerous oligomers, annular protofibrils, lipoprotein complexes, and transmembrane channels contain symmetric β-barrels in which all monomers have identical conformations. These models are consistent with well-established β-barrel theory, molecular modeling principles, and experimental data, including microscopic images.

Conclusions: Hypothetical models and concepts are vital for research because they may suggest experiments that otherwise would not be performed or funded. These experiments include methods: to test aspects of the models, to reduce polymorphism and disorder so that specific structures can be solved experimentally, and to identify specific segments to target for drug and antibody treatments.
PROTECTIVE ROLE OF AB MONOMERS AND SOME OF ITS PEPTIDE FRAGMENTS IN HANDLING PHYSIOLOGICAL COPPER

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**Aims:** Amyloid-β as monomeric species are essential players in neuronal survival and synaptic function regulation, while Aβ oligomers are pathogenic markers of Alzheimer’s disease (AD). Different studies indicate that the Aβ plaques toxicity is also mediated by biometals, Cu, in particular, altering the intracellular metallostasis of AD brains, while the protective role of Aβ monomers in handling transition metal ions as copper is less investigated. Here, we report on the effects of physiological amount of copper intake by means of Aβ and some of its peptide fragments.

**Methods:** Primary neuronal cultures and differentiated SH-SY5Y cells were used as cellular models in the study. By the use of western blot analysis, we investigated the effect of copper ions on the abilities of Aβ fragments to promote Trk receptors and CREB phosphorylation and to affect the cell metallostasis. Fluorescence imaging, flow cytometry and immunofluorescence were carried out to evaluate the cellular uptake of fluorescent Aβ peptides and to determine their effects on the intracellular translocation of copper transporters/chaperones. ELISA was used to investigate the BDNF protein release.

**Results:** We found that the ionophore ability of Ab tunes Trk signaling pathways, including the induction of Trk receptors, CREB phosphorylation and BDNF release. Furthermore, our study shows that several metallostasis players (Ctr1, Sp1, CCS, Atox1) are involved in Aβ activity.

**Conclusions:** Based on our data, the involvement of Trk signaling pathways and metallostasis in AD may be considered as a promising target for pathophysiology of AD progression.
AMYLOID-B OLIGOMERIZATION STUDY USING NOVEL COMPUTATIONAL PROTOCOLS

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Aims: The oligomerization of amyloid-β (Aβ) peptides is a major neuropathological hallmark of Alzheimer’s disease (AD). Although Aβ oligomerization has been intensively investigated in both theoretical and experimental studies, its kinetics and molecular mechanism remain mysterious to us. In this study, we developed and applied novel computational protocols to investigate the oligomerization of Aβ peptides at different conditions including monomer concentrations, mutations, and the presence of ligands.

Methods: We utilized molecular dynamics (MD) simulations of Aβ oligomerization. We estimated the oligomerization time of dimer, trimer, and tetramer; we tracked oligomerization pathways; we characterized the structures of the Aβ oligomers; we analyzed intermolecular residue-residue interactions in the different oligomers.

Results: We have established for the first time derived equations that could quantitatively describe the relationship between the oligomerization time and the monomer concentration. We’ve found that the Aβ42 oligomerization time depends on the monomer concentration by a power of -2.4. The residue-residue interaction analysis showed that K28 residues play an important role in the formation of Aβ42 oligomers. K16F/E22F mutations speeded up the oligomerization process of Aβ16-22 peptides.

Conclusions: Our established equations quantitatively can estimate the risk score of AD, which is a function of age. It also indicated that Aβ42 tetramer is the critical nucleus of the early Aβ42 oligomerization. Additionally, we have identified the most dominant pathway of forming Aβ tetramers, probably the most important and toxic Aβ oligomer. The increase of the peptide hydrophobicity speeds up the oligomerization process.
ON-DEMAND SYMPOSIUM: AMYLOIDS, TAU ALPHA-SYNUCLEIN, PRIONS. PROTEIN MISFOLDING; DISEASE MECHANISMS & DETECTION 2

NEW APPLICATIONS OF DIFFUSION MODEL BASED PREDICTION OF PATHOLOGICAL BRAIN ALTERATIONS: INTRODUCING AMYLOID-TAU INTERACTIONS

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Aims: There is mounting evidence for "prion-like" trans-neuronal transmission of misfolded tau and Ab proteins along white matter projections in Alzheimer's disease. It is not well understood how tau and amyloid beta interact, and how their subsequent spread lead to stereotypical progression in the Alzheimer brain. To explore this interaction and its brain-wide effect in silico, we present a major, advancement of our prior Network Diffusion Model (NDM) (Raj et al, Neuron 2012). This extended eNDM model enables in silico exploration of cross-species interactions and differential network spread in human brains.

Methods: eNDM incorporates three key processes: a) Tau monomer seeding in entorhinal cortex, and Ab monomer seeding in metabolically active areas. b) Interactions between Ab and tau, e.g. Ab facilitates production of tau. c) Both species then undergo network spread mediated by anatomic fiber connections, via the NDM model. The eNDM was thoroughly tested on empirical regional MRI-derived atrophy, AV45-PET and AV1451-PET from ADNI-3 (Table 1).

Table 1. Sample size of the ADNI-3 diagnostic cohorts used in this study. AD: Alzheimer's Disease patients, EMCI: early mild cognitive impairment, LMCI: late MCI, HC: healthy controls. These subjects represent the most complete set of subjects included in the ADNI-3 study as of 1/1/2020 who had all 3 imaging data (AV45-PET, AV1451-PET and MRI).

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>N</th>
<th>Age range</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>68</td>
<td>73.8 (8.12)</td>
</tr>
<tr>
<td>EMCI</td>
<td>137</td>
<td>72.1 (5.91)</td>
</tr>
<tr>
<td>LMCI</td>
<td>75</td>
<td>71.9 (7.61)</td>
</tr>
<tr>
<td>HC</td>
<td>251</td>
<td>74.10 (6.69)</td>
</tr>
</tbody>
</table>

Results: The extended NDM exhibits all hallmarks of tau and amyloid progression in patients visually (Fig1), and numerically (Table 2). Remarkably, introduction of 1-way Aβ to tau influence (Fig1 middle) performed best and recapitulated the concept of amyloid-facilitated tauopathy. In comparison, non-interacting model (right) was significantly worse, while 2-way (bidirectional) model was essentially the same (see Table 2).
Table 2: Peak Pearson’s R of correlation between model and empirical regional statistics. Highly significant correlations: ** (p < 0.001), moderately significant: * (p < 0.01), corrected for multiple comparisons. Three theoretical model evaluations are presented: the No-interaction model, without the interaction term (); the 1-way interaction model, whereby amyloid affects tau but not vice versa; and the 2-way interaction model where both tau and amyloid affect each other. Fisher’s R-to-z transform was computed, and the p-value of significant differences between each model-pair was evaluated. P-values that are smaller than the significance threshold (p<0.05) are highlighted in bold red font, and indicate comparisons where one model is significantly different than another. Aikeke information criterion (AIC) is reported for each model in brackets. The lowest AIC is always achieved by the 1-way interaction model, as well as the highest R overall.

<table>
<thead>
<tr>
<th>Empirical data</th>
<th>No-interaction Model</th>
<th>1-way interaction Model (Aβ enhances tau)</th>
<th>Fisher R-to-z, no-int vs 1-way int</th>
<th>2-way int Model (mutual synergy between Aβ, tau)</th>
<th>Fisher R-to-z, 1-way vs 2-way int</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMCI Aβ</td>
<td>0.61 **</td>
<td>0.61 **</td>
<td>p = 1</td>
<td>0.61 **</td>
<td>p = 1</td>
</tr>
<tr>
<td>LMCi Aβ</td>
<td>0.53 **</td>
<td>0.53 **</td>
<td>p = 1</td>
<td>0.53 **</td>
<td>p = 1</td>
</tr>
<tr>
<td>AD Aβ</td>
<td>0.46 *</td>
<td>0.46 *</td>
<td>p = 1</td>
<td>0.46 *</td>
<td>p = 1</td>
</tr>
<tr>
<td>EMCI tau</td>
<td>0.46 * (-28.5)</td>
<td>0.58 ** (-38.7)</td>
<td>p &lt; 0.01</td>
<td>0.56 ** (-35.0)</td>
<td>p = 0.30</td>
</tr>
<tr>
<td>LMCi tau</td>
<td>0.55 ** (-3.7)</td>
<td>0.66 ** (-17.2)</td>
<td>p &lt; 0.01</td>
<td>0.65 ** (-13.6)</td>
<td>p = 0.40</td>
</tr>
<tr>
<td>AD tau</td>
<td>0.44 * (84.5)</td>
<td>0.51 * (80.6)</td>
<td>p = 0.09</td>
<td>0.52 * (81.0)</td>
<td>p = 0.40</td>
</tr>
<tr>
<td>EMCI atrophy</td>
<td>0.29 (-23.1)</td>
<td>0.40 * (-31.3)</td>
<td>p = 0.025</td>
<td>0.37 * (-25.2)</td>
<td>p = 0.25</td>
</tr>
<tr>
<td>LMCi atrophy</td>
<td>0.51 * (-26.4)</td>
<td>0.58 ** (-33.0)</td>
<td>p = 0.080</td>
<td>0.56 ** (-28.2)</td>
<td>p = 0.30</td>
</tr>
<tr>
<td>AD atrophy</td>
<td>0.49 * (110)</td>
<td>0.60 ** (94.5)</td>
<td>p = 0.013</td>
<td>0.57 ** (103)</td>
<td>p = 0.22</td>
</tr>
</tbody>
</table>
Conclusions: eNDM captures a “pas de deux” of co-evolving proteins, and supports a key role for amyloid-facilitated-tau rather than the classic amyloid-cascade or pure-tau hypotheses, and helps explain known but poorly understood aspects of AD (Figure 2).

Figure 2: Model of spatiotemporal progression of AD-related amyloid (red) and tau (blue). Following diffuse production of amyloid in proportion to metabolism, and focal production of tau at the ERC, aggregation into plaques and tangles occur at networked sites following graph topology. Tau pathology is further aggravated by the arrival of amyloid in temporal areas. Finally the classic AD spatial patterns are established - frontal-dominant for amyloid and temporal dominant for tau.
ON-DEMAND SYMPOSIUM: AMYLOIDS, TAU ALPHA-SYNUCLEIN, PRIONS. PROTEIN MISFOLDING; DISEASE MECHANISMS & DETECTION 2

PROPAGATION OF AMYLOID PATHOLOGY BY CELL-DERIVED Aβ HEXAMERIC SEED

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Aims: The formation of Aβ assemblies plays a crucial role in AD pathology. Our aim was to characterize pathological Aβ oligomers produced by cells and investigate their seeding properties and their contribution to amyloid pathology in a FAD mouse model.

Methods: Immunoprecipitation combined to mass spectrometry was used for the characterization of Aβ assemblies produced by cells expressing human APP amyloidogenic fragments (e.g C99 or βCTF). Nucleation properties of cell-derived Aβ assemblies were studied in vitro. Knock-down of Presenilin1/2 was performed to identify the type of γ-secretase involved in their production. Hexameric Aβ assemblies were isolated from cell cultures and further injected in FAD mice to study their pathological properties.

Results: Aβ hexamers were readily detectable in cell extracts and culture media of various cell lines expressing APP amyloidogenic fragments. Hexameric Aβ42 produced by cells displayed nucleating properties which are dependent on the availability of Aβ monomers. We found that Aβ hexamers were predominantly associated to PS2-dependent γ secretases. Hexameric-like Aβ assemblies were detected both in FAD mice brains and in the cerebrospinal fluid of AD patients. Finally, cell-derived hexameric Aβ was able to seed other human Aβ forms, resulting in the aggravation of amyloid deposition in FAD mice.

Conclusions: Aβ42 hexamers produced by cells have intrinsic nucleation features underlying their pathological properties. The PS2-dependent γ-secretase is critical for their production. They are present in FAD mice brains and human fluids, and their seeding properties aggravate the amyloid pathology in FAD mice.
HUMAN ISLET AMYLOID POLYPEPTIDE AND METAL BINDING: AN INVESTIGATION INTO AMYLOID PROTEIN AGGREGATION IN THE PRESENCE OF METAL IONS

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Aims: Human islet amyloid polypeptide (hIAPP) aggregation is a hallmark of type-2 diabetes, a risk factor for development of Alzheimer’s disease, and has been observed in brain tissue and associated with cognitive decline. This study aimed to monitor the impact of physiologically abundant metals (iron, copper, magnesium, calcium) and trace elements such as manganese and cobalt, on hIAPP aggregation.

Methods: Time-of-flight mass spectrometry (TOF-MS) can precisely analyse samples and separate species by mass. Micromolar concentrations of hIAPP were co-incubated with a range of metal ions and sampled regularly over a one hour period for TOF-MS analysis.

Results: Aggregation occurred through conversion of early-stage oligomers into fibrils; as this proceeded faster than initial monomer-dimer conversion, monitoring these rates enabled the aggregation process to be quantified and tracked. Apoprotein analysis showed a decreasing dimer amount as incubation progressed, indicating that aggregation gained momentum between ‘monomer-dimer’ and ‘dimer-oligomer’ conversion. Spectral features indicated unique values and corresponding species that distinguished each sample condition. Impacts of metal co-incubation included copper binding to hIAPP, with a decreased conversion rate compared to apoprotein, cobalt binding that diminished as incubation progressed, and ferrous (but not ferric) iron binding to hIAPP.

Conclusions: Through TOF-MS analysis and quantification, we were able to accurately monitor the dynamics of hIAPP aggregation with great precision, examining the influence of physiologically relevant metals. The work advances understanding of hIAPP aggregation, and the protocols developed for this work are now being adapted to enable study TOF-MS analysis of other peptides of interest in Alzheimer’s and Parkinson’s disease, including α-synuclein.
Aβ AND TAU OLIGOMERS SHOW DIFFERENTIAL SYNAPTIC ENGAGEMENT SUGGESTING DISTINCT SYNAPTIC TOXICITY ACROSS ALZHEIMER'S DISEASE AND RELATED TAUOPATHIES

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Aims: Alzheimer’s Disease (AD) is characterized by gradual cognitive decline due to accumulating synaptic insults by toxic oligomers of amyloid beta (AβO) and tau (TauO). Current research focuses on understanding how to prevent their interaction with synapses as an effective therapeutic approach for AD. With this goal in mind, here we show that recombinant TauO, human brain derived tau oligomers (BDTOs) as well as AβO, target synapses with different dynamics.

Methods: Binding and internalization of labeled, pre-formed oligomers onto synaptosomes from mouse and human brains by using flow-cytometry, western blot, immunofluorescence analyses, and proteinase K (PK) digestion; synaptic dysfunction measured by FASS-LTP assay.

Results: We demonstrated that higher TauO concentrations outcompete AβO and become the prevailing synaptic-associated species, while high concentrations of AβO facilitate synaptic TauO recruitment. These findings were confirmed by in vivo experiments using old 3xTgAD mice injected ICV with AβO or TauO. FASS-LTP analyses demonstrated that TauO-induced suppression of chemical LTP was exacerbated by AβO. Furthermore, predigestion with PK abolished the ability of TauO to outcompete AβO without affecting the ability of high AbO levels to increase synaptic TauO recruitment. Moreover, we observed different synaptic engagement and internalization profiles among the analyzed BDTOs, suggesting different synaptic toxicity of TauO across the tauopathies spectrum.

Conclusions: Our results indicate that at the advanced stages of AD, TauO become the main synaptotoxic species and likely an effective therapeutic target. Furthermore, subtle disease-specific differences in the dynamic of BDTOs engagement of synapses may underscore different clinical presentations among the tauopathy spectrum.
CRISPR SCREENS REVEAL REGULATORS OF TAU PATHOLOGY INDUCED BY EXOSOMAL AND VESICLE-FREE TAU SEEDS

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Aims: In Alzheimer's disease (AD), tau seeds propagate transsynaptically and corrupt the proper folding of soluble tau in recipient neurons. Tau seeds that induce tau aggregation and propagate transsynaptically are: (i) vesicle-free tau in the form of oligomers or fibrils, and (ii) exosomal tau seeds encapsulated by the membranes of exosomes. We aim to discover novel cellular regulators of tau pathology induced by these two types of tau seeds using CRISPR screens.

Methods: Tau transgenic mice were used to isolate sarkosyl insoluble vesicle-free tau, and brain-derived exosomes. Optimized genome-wide CRISP libraries were screened in tau biosensor cells to discover novel regulators of seeded tau aggregation.

Results: Our CRISPR screens revealed novel regulators of tau aggregation in cellular models. We functionally validated several of the strongest hits and revealed that individual knockdowns of each target predisposed tau biosensor cells to tau aggregation. Surprisingly, some targets (ANKLE2 and BANF1) appeared to exert a stronger effect on exosomal seeds compared to vesicle-free seeds. Interestingly, western blot analyses using brain samples from AD patients revealed that some validated hits were downregulated, suggesting that tau aggregation in AD patients may require downregulation of the discovered targets.

Conclusions: We have validated novel negative regulators that oppose the formation of tau aggregates. Some of these genes are downregulated in AD patients, which may imply a functional role in the emergence of tau pathology in humans. Further experiments will reveal how these genes regulate tau aggregation, and why some affect exosomes more strongly.
AMYGDALA VOLUME IN PROPOSED BRAIN-FIRST VS. BODY-FIRST PARKINSON’S DISEASE SUBTYPES

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Aims: Parkinson’s disease (PD) is characterized by intracellular protein aggregation of misfolded α-synuclein and subsequent neuronal degeneration. New lines of evidence suggest high interindividual heterogeneity in the sequence of locations in the brain where this pathophysiology occurs. A new hypothesis of α-synuclein spread distinguishes between two subtypes based on the initial site of the pathology: a brain-first and body-first type. In comparison to body-first, brain-first PD is conceptually associated with an earlier involvement of the amygdala, higher asymmetry of pathology, and the relative absence of REM-sleep-behavioral-disorder. The aim of our study was to test this hypothesis by examining amygdala volumes of “brain-first” and “body-first” PD subtypes.

Methods: Data of 255 PD patients were obtained from a database (ppmi-info.org). We first explored the association between asymmetry of pathology (higher putamen DaT asymmetry indicating brain-first PD) and amygdala volume of the more affected hemisphere (MAH) in PD patients. Secondly, we compared the amygdala volume of the MAH of a group of RBD+ PD patients (body-first) against a group of RBD-PD patients (brain-first).

Results: The magnitude of the putamen asymmetry was not significantly related to the amygdala volume of the MAH (r = -0.05, p = 0.43). Moreover, the amygdala of the MAH was significantly smaller in RBD+ subjects compared RBD- subjects (p =
Conclusions: Our findings appear to indicate that the proposed earlier involvement of the amygdala in brain-first PD is not reflected in a greater volume reduction. Thereby, this study fails to provide evidence in support of the brain-first/body-first spreading hypothesis.
ON-DEMAND SYMPOSIUM: AMYLOIDS, TAU ALPHA-SYNUCLEIN, PRIONS. PROTEIN MISFOLDING; DISEASE MECHANISMS & DETECTION 2

ALPHA-SYNUCLEIN PHOSPHORYLATION AT SERINE 129 OCCURS AFTER INITIAL PROTEIN DEPOSITION AND INHIBITS SEEDED FIBRIL FORMATION AND TOXICITY

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Aims: α-Synuclein (α-syn) phosphorylation at serine 129 (pS129-α-syn) is substantially increased in Lewy body disease, such as Parkinson’s disease (PD) and dementia with Lewy bodies (DLB). However, the pathogenic relevance of pS129 modification remains controversial, so we sought to identify when pS129 modification occurs during α-syn aggregation and its role in initiation, progression and cellular toxicity of disease.

Methods: Using diverse aggregation assays, including real-time quaking-induced conversion (RT-QuIC) on brain homogenates from PD and DLB cases, the effect of pS129 modification on α-syn fibril formation, seeding aggregation, and cellular toxicity was monitored. Using our novel monoclonal antibody (4B1) specifically recognizing non-phosphorylated S129-α-syn (WT-α-syn), we characterized the time-course of α-synuclein phosphorylation in organotypic mouse hippocampal cultures, mice injected with α-synuclein preformed fibrils and post-mortem brain tissue from PD and DLB patients.

Results: We demonstrated that pS129-α-syn inhibits α-syn fibril formation and seeded aggregation, lowers seeding propensity in cultured cells and attenuates cellular toxicity. We observed aggregation of non-phosphorylated α-synuclein followed by pS129-α-syn in ex vivo and in vivo models. In post-mortem brain tissue, we noted an inverse relationship between relative abundance of non-phosphorylated α-synuclein and disease duration, potentially implying increasing phosphorylation over time.

Conclusions: These findings suggest that pS129-α-syn occurs subsequent to initial protein aggregation and inhibits further aggregation. This may imply a potential protective role for pS129-α-syn, which has major implications for understanding the pathobiology of Lewy body disease and the continued use of reduced pS129-α-syn as a measure of efficacy in clinical trials.
ON-DEMAND SYMPOSIUM: AMYLOIDS, TAU ALPHA-SYNUCLEIN, PRIONS. PROTEIN MISFOLDING; DISEASE MECHANISMS & DETECTION 2

DTI ABNORMALITIES IN HEALTHY E200K CARRIERS MAY SERVE AS AN EARLY BIOMARKER FOR GENETIC CREUZFELDT-JAKOB DISEASE (GCJD)

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Aims: To investigate microstructural changes in healthy E200K carriers using diffusion tensor imaging (DTI).

Methods: Seven symptomatic gCJD patients and N=60 healthy relatives of gCJD patients were included. Participants underwent genetic testing for the E200K mutation, MRI scans at 3T, and a lumbar puncture (LP) for total Tau protein levels (t-Tau). Diffusion tensor imaging (DTI) metrics including; fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD) and axonal diffusivity (AD) were calculated along 45 WM tracts.

Results: N=30 participants were found to be E200K carriers (mean age 56.73±7.27, 18F). Of those; N=23 underwent an LP, and 8 showed above normal t-Tau values (> 290 pg/ml). Higher MD, RD as well as lower FA and AD values were observed in symptomatic CJD patients compared to healthy relatives in several WM tracts (Two-samples t-test; p<0.05). Non-carriers demonstrated higher FA in the right anterior and posterior limbs of internal capsule compared to carriers (Two-samples t-test; p<0.05). Finally, significantly higher FA and lower MD, RD, and AD were found in carriers with high level of t-Tau compared to carriers with normal levels of t-Tau along several WM tracts.

Conclusions: DTI abnormalities along WM tracts were found in healthy E200K carriers with elevated t-Tau in CSF. These findings suggest a possible role for DTI imaging as a non-invasive biomarker for prodromal gCJD. Ongoing work is focused on identifying DTI measures and additional candidate MRI markers prior to phenoconversion.
THE AB1-42/AB1-40 RATIO IN CSF IS MORE STRONGLY ASSOCIATED TO TAU MARKERS AND CLINICAL PROGRESSION THAN AB1-42 ALONE

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Aims: Cerebrospinal fluid (CSF) Aβ1-42 levels and the Aβ1-42/Aβ1-40 ratio are markers of amyloid pathology in Alzheimer’s disease (AD), but previous studies suggest that other pathophysiological processes might distinctly influence their levels. We aimed to compare Aβ1-42 and the Aβ1-42/Aβ1-40 ratio in CSF in different neurodegenerative disorders and to study their association with other CSF biomarkers (tTau, pTau181 and NfL) and with cognitive and functional progression.

Methods: We included 1791 participants from the Sant Pau Initiative on Neurodegeneration (SPIN). Participants had diagnoses of AD, dementia with lewy bodies, frontotemporal lobar degeneration-related syndromes, non-neurodegenerative conditions, or were cognitively normal. We classified participants as “positive” or “negative” according to each marker cutoff. We compared CSF levels of tTau, pTau181 and NfL between groups through ANCOVA and assessed differences in cognitive (MMSE, FCSRT) and functional (GDS, CDR-SOB) progression using Cox regression and linear-mixed models.

Results: In the 1791 participants, the agreement between Aβ1-42 and Aβ1-42/Aβ1-40 was 78.3%. Aβ1-42/Aβ1-40 ratio showed stronger correlation with tTau and pTau181 than Aβ1-42 (Figure 1). Both within the Aβ1-42 negative group and the Aβ1-42 positive group, participants with low Aβ1-42/Aβ1-40 ratio showed higher levels of tTau and pTau181 and worse cognitive and functional prognosis than participants with normal ratio (Figure 2). Results were consistent across clinical stages, diagnostic categories and use of different cutoffs.

Conclusions: Although Aβ1-42 and Aβ1-42/Aβ1-40 are considered markers of the same pathophysiological pathway, our findings provide evidence favoring the use of the Aβ1-42/Aβ1-40 ratio in clinical laboratories in the context of AD.
Aims: To study associations between plasma p-tau217 (which is one of the most promising blood biomarkers for sporadic Alzheimer’s disease [AD]) and amyloid (Aβ) and tau positron emission tomography (PET) measures in individuals with Down syndrome (DS).

Methods: We analyzed p-tau217 in baseline plasma samples from 301 participants with DS and 37 non-DS sibling controls using MSD immunoassay from Lilly. Plasma NfL was quantified with Simoa immunoassay. Aβ-PET and tau-PET data were available for 163 and 79 adults with DS (35 years of age and older), respectively.

Results: Plasma p-tau217 levels were increased in DS compared to the non-DS sibling control group (Figure). In Aβ-positive DS, plasma p-tau217 levels correlated strongly with tau-PET signal in AD susceptible brain regions (entorhinal cortex r=0.67, temporal meta-ROI r=0.80; neocortical meta-ROI r=0.72; p<0.001), but not in Aβ-negative DS. In participants with DS, plasma p-tau217 accurately detected tau-PET positivity (AUC range 0.86-0.97) performing better than plasma NfL (AUC range 0.78-0.84). Plasma p-tau217 had high discriminative accuracy (AUC=0.91) for Aβ-PET status (normal vs abnormal, Figure) while plasma NfL showed inferior performance (AUC=0.80). In multivariable logistic regression models with plasma p-tau217, plasma NfL and age as predictors and either Aβ-PET or tau-PET as outcome, plasma p-tau was significantly associated with both Aβ-PET or tau-PET whereas NfL was not significant.

Conclusions: Plasma p-tau217 is a promising biomarker of AD-related Aβ and tau pathologies in DS that could help guide screening and enrichment strategies and facilitate inclusion of people with DS in future AD clinical
trials.
Aims: Plasma biomarkers of amyloid and neurofilament light chain (NfL) provide a more accessible and cost-effective alternative to cerebrospinal fluid (CSF) markers. We aimed to validate the use of plasma biomarkers by comparing plasma and CSF biomarkers as predictors of cognitive decline in a cohort of cognitively healthy participants.

Methods: 561 (mean age = 68, range = 43 – 91) clinically normal participants were analyzed for this study. All had at least two cognitive assessments and baseline measurements of amyloid and NfL in both plasma and CSF. Plasma Aβ42 and Aβ40 were measured with a commercial immunoprecipitation-mass spectrometry assay. Plasma NfL was measured with the Quanterix Simoa assay. CSF Aβ42 and Aβ40 were measured with the automated Lumipulse platform. CSF NfL was measured with the Uman ELISA. Our primary outcome was rates of change on three cognitive composite scores: global cognition, memory and attention. Linear mixed effects models were used to compare baseline effects and rates of change in cognition associated with each biomarker.

Results: There were no baseline associations between cognition and any of the four biomarkers. However, abnormal levels of Aβ42/Aβ40 and NfL in both CSF and plasma were independently associated with faster rates of cognitive decline. Participants with abnormal levels of both Aβ42/Aβ40 and NfL markers showed the fastest rates of decline.

Conclusions: Plasma measures of amyloid and NfL are valid predictors of future cognitive change, even among participants who are clinically healthy at baseline, and can serve as a more accessible and cost-effective biomarkers than CSF measures.
ON-DEMAND SYMPOSIUM: AD: DIAGNOSIS, CLINICAL TRIALS & ADVANCES IN TRANSLATIONAL DRUG DEVELOPMENT

INTEGRATIVE EVALUATION OF EARLY ALZHEIMER PATHOLOGY WITH QUANTITATIVE GRADIENT RECALLED ECHO (QGRE) MRI AND PLASMA BIOMARKERS

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Aims: The A/T/N framework allows staging of Alzheimer Disease (AD) based on the interplay of brain amyloidosis, tauopathy and loss of neurons, the latter usually defined by MRI-based measurement of tissue atrophy. Herein we hypothesize that combining two new biomarkers – plasma amyloid (PrecivityAD™) and MRI-based quantitative-Gradient-Recalled-Echo (qGRE) measurements of tissue pre-atrophic neurodegeneration — will allow for classification of early AD.

Methods: qGRE and plasma amyloid measurements were obtained from 31 participants (13 healthy control, 12 preclinical AD and 6 early symptomatic AD based on Clinical Dementia Rating and CSF amyloid status). PrecivityAD™ uses liquid chromatography-mass spectrometry to measure plasma Aβ42/Aβ40 and Apolipoprotein-E isoform-specific peptides. Along with age, these measures are incorporated into a model that predicts brain amyloid PET status and assigns the Amyloid Probability Score (APS); the likelihood of brain amyloid positivity. qGRE separates tissue in two components – Viable Tissue (VT) with slightly altered concentration of neurons and tissue essentially devoid of neurons (Dark Matter, DM).

Results: ROC classification tree results based on qGRE metrics (DM and VT Volumes) in the hippocampus and plasma APS are presented in Figure 1 showing significantly improved classification using all three biomarkers (p < 0.05).
Conclusions: Integrating qGRE metrics with a blood amyloid biomarker results in clinically important improvements in individual qGRE and blood amyloid tests regarding sensitivity and specificity for an early detection of AD pathology. Because both tests (qGRE and APS) can be performed in a traditional clinical setting, this approach has a great potential for broad applications in early AD detection.
ON-DEMAND SYMPOSIUM: AD: DIAGNOSIS, CLINICAL TRIALS & ADVANCES IN TRANSLATIONAL DRUG DEVELOPMENT

PLASMA NEUROFILAMENT LIGHT AS A MARKER OF PSYCHOSIS IN PARKINSON'S DISEASE

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Aims: Neuropsychiatric symptoms (NPS) are common to Parkinson’s disease (PD) but their aetiology is poorly understood. Plasma neurofilament light (NFL) is a biomarker of neuroaxonal degeneration which has yet to be explored in association with the NPS in PD. We aimed to investigate the longitudinal relationship between NFL and psychotic and affective symptoms in PD.

Methods: We evaluated the association of NPS with NFL in a cohort (n = 144; PD = 106, healthy controls (HC) = 38). Plasma NFL was measured at baseline and NPS were measured with Non-Motor Symptom Scale (NMSS) for psychotic symptoms and Hospital Anxiety and Depression Scale (HADS) for affective symptoms. A subset of patients (n=61) were assessed annually for NPS. NPS were quantified dichotomously on a cumulative basis as they were developed during the study.

Results: 73 (86%) PD patients developed NPS at some time point in the study; 37 with psychotic symptoms and 70 with affective symptoms. Plasma NFL was elevated in patients who developed psychotic symptoms in age-adjusted logistic regression (OR 3.81 [1.06-13.7], p=0.04). There was no association between NFL concentration and affective symptoms.

Conclusions: These findings suggest cumulative psychotic symptoms are associated with greater neurodegeneration in PD. It suggests differing neurobiological aetiologies underpinning the psychotic and affective symptoms seen in PD. Further studies are needed to explore NFL as a potential prognostic biomarker for psychotic symptoms in PD.
ON-DEMAND SYMPOSIUM: AD: DIAGNOSIS, CLINICAL TRIALS & ADVANCES IN TRANSLATIONAL DRUG DEVELOPMENT

A COMBINED IN SILICO/IN VITRO APPROACH UNVEILS DRUG REPURPOSING CANDIDATES TARGETING ALZHEIMER PATHOPHYSIOLOGY MECHANISM

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Aims: The high number of failed pre-clinical and clinical studies for compounds targeting Alzheimer’s Disease (AD) has demonstrated that there is a need to reassess existing strategies. Here, we pursue a holistic, mechanism-centric drug repurposing approach combining computational analytics and experimental screening data. The main objective of our joint approach is to suggest highly relevant drug repurposing candidates for testing in clinical trials in the context of AD.

Methods: We combine drug-target information with knowledge graphs that represent essential pathophysiology mechanisms. The resulting Human Brain PHARMACOME (HBP) embeds all relevant drug-target interactions in the context of a massive collection of computable disease mechanisms underlying Alzheimer’s Disease.

Results: To demonstrate its utility, we used the HBP to identify upstream controllers of posttranslational modification of the Tau protein, with a strong focus on phosphorylated Tau (pTau), one of the hallmarks of Alzheimer’s Disease. Interestingly, HDAC6 was identified as one of the pleiotropic regulators controlling posttranslational modification of Tau. In a dedicated drug repurposing (DR) approach, we established a HDAC6 – Tau assay and screened a repurposing library consisting of 5632 approved drugs for compounds that modulate Tau phosphorylation. We identified, profiled and validated 20 compounds that indeed modify pTau through HDAC6. Four compounds, and their known targets, were found to have a link to AD specific genes.

Conclusions: We identified highly interesting repurposing candidates and new drug-target combinations and provided mechanistic explanations that help to improve our understanding of AD pathology and support future development of effective therapeutic strategies.
Aims: Innate immune Aβ clearance may be involved in Alzheimer’s disease (AD) etiology. The molecular mechanism regulating Aβ clearance are unclear. The aims of the present work were to first determine which transcripts are differentially expressed, and which relevant molecular pathways are affected by docosahexaenoic acid (DHA)+NS-1738 (α7 nAChRs positive allosteric modulator, PAM) combination that we had earlier shown increase Aβ clearance, and further to describe perturbagens targeting these pathways.

Methods: THP-1 cells were treated with DHA+NS and were processed for the Aβ degradation assay. RNA was isolated for transcriptomics (Novogene). From the transcriptomics data, the top 10 differentially regulated genes (based on log2fold change), either unfiltered or filtered on the basis of gene ontology (KEGG pathways involved in aggregate clearance or neuroinflammation) were used to explore perturbagens which can induce effects similar to DHA+NS using CMAP database. A list of perturbagens rated according to their net “beneficial” effect on the top differentially modulated was produced.

Results: 1. Catabolism of Aβ1-40 is enhanced in DHA+NS treatment in THP1 cells. 2. The DHA+NS treatment mainly influences pathways related to endo-lysosomal function and neuroinflammation. 3. Lists of perturbagens working in a manner similar to DHA+NS were produced.

Conclusions: 1. We demonstrate a novel paradigm to identify potential modulators of Aβ clearance. 2. Both the endo-lysosomal machinery and the innate immune response are modulated showing links to Aβ clearance and thus putative druggable targets. 3. As a proof of concept, some of the compounds identified using this transcriptomics-based approach are already in clinical trials against AD.
Aims: Synaptic dysfunction plays a central role in Alzheimer's disease (AD), since it drives the cognitive decline. An association between a polymorphism of the adenosine A2A receptor (A2AR) encoding gene—ADORA2A, and hippocampal volume in AD patients was recently described. We now explored the role of A2AR in hippocampal function in age-related conditions.

Methods: We showed a significant upsurge of A2AR in hippocampal neurons of aged humans, a phenotype aggravated in AD patients. Increased expression of A2AR driven by the CaMKII promoter selectively in rat forebrain neurons was sufficient to mimic aging-like memory impairments and to uncover a LTD-to-LTP shift in the hippocampus.

Results: We report a significant overexpression of A2AR in hippocampal neurons of aged humans, which is aggravated in AD patients. A similar profile of A2AR overexpression in young rats was sufficient to drive age-like memory impairments and to uncover a LTD-to-LTP shift. This shift was due to an increased NMDA receptor gating and associated to increased Ca2+ influx. We identified the mGluR5-NMDAR interplay as key player in the observed A2AR-induced synaptic dysfunction. Importantly, the same LTD-to-LTP shift was observed in memory-impaired aged rats and APP/PS1 mice modeling AD, a phenotype rescued upon A2AR blockade.

Conclusions: Due to the aberrant A2AR signaling in pathological conditions, their blockade is particularly relevant for long-term therapies, since the alternative option of targeting directly either mGluR5 or NMDAR interferes with basal neuronal function and memory, as these proteins are crucial components of the postsynaptic density.
ON-DEMAND SYMPOSIUM: AD: DIAGNOSIS, CLINICAL TRIALS & ADVANCES IN TRANSLATIONAL DRUG DEVELOPMENT

TREM2-INDUCED ACTIVATION OF MICROGLIA CONTRIBUTES TO SYNAPTIC RESILIENCE IN NON-DEMENTED INDIVIDUALS WITH ALZHEIMER'S NEUROPATHOLOGY

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Aims: The existence of individuals who remain cognitively intact despite presenting histopathological signs of Alzheimer's disease (AD), here referred to as "Non demented with AD neuropathology" (NDAN), suggests that some mechanisms are triggered to resist cognitive impairment. Exposed phosphatidylserine (ePS) represents a neuronal "eat-me" signal involved in microglial-mediated phagocytosis of damaged synapses. A possible mediator of this process is TREM2, a microglial surface receptor activated by ligands including PS. Based on TREM2 role in the scavenging function of microglia, we hypothesize that an efficient microglial phagocytosis of damaged synapses underlies synaptic resilience in NDAN, thus protecting from memory deficits.

Methods: Using immunofluorescence microscopy, we performed a comparative study of human post-mortem frontal cortices of aged-matched, AD and NDAN individuals. The distribution of activated microglia (IBA1 and IBA1+/CD68+ positive cells) and microglia-related proteins (TREM2 and DAP12) were evaluated. Furthermore, to test the efficacy of microglia in removing damaged synapses, preservation of synapses around plaques was assessed using MAP2 and tubulin βIII as dendritic and axonal markers, and PSD95 as a postsynaptic marker. Furthermore, using flow cytometry, a study of the ePS using Annexin V assay has been performed.

Results: NDAN show higher microglial activation and TREM2 expression, as well as preserved axonal and dendritic structure around plaques vs. AD. Higher levels of PSD95 and ePS around NDAN plaques may suggest a prompt removal of damaged synapses by efficient microglia.

Conclusions: Our results suggest a higher efficiency of TREM2-induced phagocytic microglia in removing damaged synapses, underlying synaptic resilience in NDAN individuals.
ON-DEMAND SYMPOSIUM: AD: DIAGNOSIS, CLINICAL TRIALS & ADVANCES IN TRANSLATIONAL DRUG DEVELOPMENT

ALPHA-SYNUCLEIN (AUTO)ANTIBODIES IN PARKINSON'S DISEASE – A FRIEND OR FOE

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\textbf{Aims:} Alpha-synuclein autoantibodies have been widely reported in the serum of the general population. Furthermore, immunoglobulins have been detected in the CSF and substantia nigra of deceased PD patients suggesting an impairment of the blood-brain barrier (BBB) in PD. Peripheral alpha-synuclein autoantibodies could potentially bypass the compromised BBB. This study aims to understand the (patho)physiological role of alpha-synuclein autoantibodies in PD progression.

\textbf{Methods:} Rat primary neuron-astrocyte cocultures or astrocyte-enriched cultures overexpressing synuclein isoforms were exposed to human serum containing alpha-synuclein autoantibodies or anti-alpha synuclein antibodies. The effects on cell survival, network activity and chemokine release were studied using immunofluorescence, western blot, calcium imaging and cytokine bead assay. Significance was tested using one-way ANOVA with a stringency of 80% power.

\textbf{Results:} Alpha-synuclein autoantibodies significantly abrogated neuronal survival and network activity specifically in alpha-synuclein overexpressing cells in a dose-dependent manner. A similar loss of neurons was observed using anti-alpha-synuclein antibodies. Removal of alpha-synuclein autoantibodies from the serum rescued neurotoxicity. Interestingly, alpha-synuclein (auto)antibodies did not affect astrocyte survival or morphology. However, significant upregulation of one specific chemokine was observed upon exposure of the cultures to alpha-synuclein (auto)antibodies. Downstream targets of this chemokine that might cause the observed neurotoxicity and compromised network activity are currently being examined.

\textbf{Conclusions:}

Alpha-synuclein (auto)antibodies recognizing the monomeric alpha-synuclein result in a loss of alpha-synuclein overexpressing neurons but not astrocytes. We hypothesize that this neurotoxicity is potentially mediated by a chemokine secreted by astrocytes. This study hint towards a novel immune-regulated mechanism of PD.
ON-DEMAND SYMPOSIUM: AD ANIMAL MODELS AND MECHANISTIC & TRANSLATIONAL ASPECTS 1

CHRONIC EXPOSURE TO LOW DOSES OF FUNGICIDES EXACERBATES ALZHEIMER’S DISEASE MARKERS

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Aims: Pesticide residues have contaminated our environment and nutrition over the last century. Although these compounds are present at very low concentrations, their long-term effects on human health is of concern. The link between pesticide residues and Alzheimer’s disease is not clear and difficult to establish. Thus, we investigated the impact of this chronic contamination on the pathological markers of Alzheimer’s disease in a transgenic mouse model.

Methods: Transgenic (J20, hAPPSw/Ind) mice were chronically exposed to a cocktail of fungicide residues at 0.1 μg/L in their drinking water for 9 months. The dose applied corresponds to the maximal regulatory limit dose allowed in drinking water. The effects of pesticide residues were assessed on the pathological markers of the disease. Then, we studied the dynamics of Aβ aggregation in vivo via a longitudinal study using 2-photon microscopy.

Results: We found that a chronic exposure to three fungicide residues exacerbated aggregation, microgliosis and neuronal loss. These fungicides also increased vascular amyloid aggregates reminiscent of cerebral amyloid angiopathy, between 6 and 9 months of treatment. The mechanism of action revealed an over-expression of the levels of the β-secretase cleaving enzyme (BACE1) combined with impairing Aβ clearance through nephrilisin (NEP).

Conclusions: Chronic exposure of the J20 mouse model of Alzheimer’s disease to low doses of fungicides strengthened the preexisting pathological markers: neuroinflammation, β-amyloid aggregation and APP β-processing (Lafon PA et al., 2020). We hypothesize that prevention strategies towards pesticide long-term exposure may be an alternative to counterbalance the lack of treatment and to slow-down the worldwide Alzheimer’s epidemic.
ON-DEMAND SYMPOSIUM: AD ANIMAL MODELS AND MECHANISTIC & TRANSLATIONAL ASPECTS 1

XENON GAS TREATMENT TO RESTORE MICROGLIAL FUNCTIONS IN ALZHEIMER'S DISEASE

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Aims: Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder. Emerging evidence shows that dysregulation of microglia plays a significant role in the onset and progression of AD. Our group recently identified a microglia transition between homeostatic (M0) to neurodegenerative (MGnD) signature, during AD progression. Xenon (Xe) gas is currently used in human patients as an anesthetic and as a neuroprotectant in the treatment of brain injuries, however, its effects on microglia are still unknown. Our aim is to evaluate if Xe-gas treatment has a protective immunomodulatory role to restore the homeostatic microglia phenotype for AD.

Methods: APP/PS1 mice were treated with Xe 70% once a week for 2 months. After this period microglia and peripheral immune cells were evaluated by flow cytometry and scRNA-seq.

Results: We discovered that Xe-treatment via inhalation modulates microglia phenotype switch from MGnD to M0-homeostatic associated with a reduction in Ab-plaque pathology and neuroinflammation. Interesting, Xe-gas treatment directly also affect the peripheral immune response with an increase in “wound healing” signature in monocytes and neutrophils from the spleen. Moreover, multiple treatments with Xenon suppress circulating neurodegenerative neutrophils from APOE e4 mice and decrease pro-inflammatory response from neutrophils in the brain of APP/PS1 mice after 2 months of treatment. Together, scRNA-seq analyses show that microglia remain in an intermediate state during disease progression with a decrease in chemokine response.

Conclusions: In conclusion, these data provide evidence that Xe-gas treatment directly induces microglia protective functions and reduces Aβ load to treat AD.
ON-DEMAND SYMPOSIUM: AD ANIMAL MODELS AND MECHANISTIC & TRANSLATIONAL ASPECTS 1

TARGETING TRANSTHYRETIN IN ALZHEIMER’S DISEASE

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Aims: Transthyretin (TTR) has a well-established role in neuroprotection in Alzheimer’s Disease (AD). We provided in vivo evidence that TTR stabilization by the small-molecule compound iododiflunisal (IDIF) is beneficial in an AD mouse model, resulting in decreased Abeta brain burden and improved cognition. We aim to find small-molecule compounds, chaperones, that behave as IDIF, enhancing the TTR/Abeta interaction, with the final aim to discover new disease-modifying AD therapies.

Methods: Through computational approaches, we selected compounds that were assessed for their ability to bind TTR and stabilize its tetrameric conformation using in vitro biological tests. The best performing molecules were run through our in-house validated high-throughput screening ternary test, to identify those that enhance the TTR/Abeta binding. The chaperoning effect of selected compounds was confirmed by Isothermal Titration Calorimetry (ITC). Drug-response studies were finally performed with the selected compounds.

Results: We screened 53 compounds (experimental hits), and after ITC assays, we prioritized a list of TTR tetramer stabilizers as chaperones of the TTR/Abeta interaction, including IDIF (discovery phase); one investigational drug (luteolin); and 3 marketed drugs (sulindac, olsalazine and flufenamic), which could be directly repurposed for clinical use. In vivo evaluation confirmed the long-term capacity of IDIF to delay hippocampal Aβ plaque formation.

Conclusions: Small-molecule chaperones have been discovered, and one of them delayed hippocampal Aβ plaque formation in a mouse model of AD. The discovered chaperones will enable: (i) validation of TTR as a target in vivo; and (ii) selection of one repurposed drug to enter clinical trials for AD.
Aims: We recently reported that inflammaging activates monocytes to convert innate B1a cells into pathogenic 4-1BBL+TNFα+MHC-Ihigh B cells (termed 4BL cells), which then induce cytolysis of CD8+ T cells and insulin resistance in elderly humans, macaques, and mice. However, the role of these or other activated B cells in aging-associated diseases, such as Alzheimer’s disease (AD) remains unknown.

Methods: JhT mice (B6.129P2-Igh-Jtm1Cgn/J), which do not develop functional B cells in the circulation due to the immunoglobulin Jh locus deletion26, were separately bred with either congenic 3×TgAD mice (with three human genes associated with familial AD, B6;129-Psen1tm1MpmTg(APPSwe,tauP301L1Lfa/Mmjax))24,25, APP/PS1 mice (B6.Cg-Tg(APPswe,PSEN1DE9) 85Dbo/J)16 or 5×FAD mice expressing mutant human APP and PSEN1 genes (B6.Cg-Tg;APPSwFILon,PSEN1*M146L*L286V)21. The effect of B cell deficiency was assessed on Amyloid-beta plaque formation, microglial cell activation and behavioral deficits.

Results: Herein, we provide counterintuitive evidence that the AD progression requires B cells. Despite expression of the AD-fostering transgenes, the loss of B cells alone is sufficient to reduce Aβ plaque burden and disease-associated microglia. It reverses behavioral and memory deficits and restores TGFβ+ microglia, respectively. Moreover, therapeutic depletion of B cells at the onset of the disease retards AD progression in mice, suggesting that targeting B cells may also benefit AD patients.

Conclusions: Taken together, we provide evidence for a “dark” side of B cells—they exacerbate manifestation of AD-like symptoms in addition to producing potentially beneficial Aβ plaque-reducing immunoglobulins and expressing AD-ameliorating cytokines.
ON-DEMAND SYMPOSIUM: AD ANIMAL MODELS AND MECHANISTIC & TRANSLATIONAL ASPECTS 1

G PROTEIN-BIASED GPR3 SIGNALING INDUCES GLIAL ACTIVATION IN A PRECLINICAL ALZHEIMER’S DISEASE MOUSE MODEL

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Aims: The recent emergence of biased G protein-coupled receptor (GPCR) ligands, which preferentially activate G protein- or β-arrestin signaling pathways, more precisely regulate GPCR biology and is leading to development of drugs with superior efficacy and reduced side-effects in heart disease, pain management, and neuropsychiatric disorders. Although GPCRs are implicated in the pathophysiology of Alzheimer’s disease (AD), biased GPCR signaling is an unexplored area of investigation in AD. Previous work demonstrated that Gpr3 deficiency modulates amyloid-β (Aβ) pathology in AD mice. However, Gpr3⁻/⁻ mice also display several adverse phenotypes such as elevated anxiety-like behavior.

Methods: Here, we generated a G protein-biased GPR3 model (Gpr3HA-Ala) to investigate the physiological and pathophysiological consequences of selective elimination of β-arrestin signaling via GPR3.

Results: Gpr3HA-Ala mice do not display anxiety-like behavior or memory deficits as observed in Gpr3⁻/⁻ mice. GPR3 is most abundantly expressed in astrocytes and displays similar levels in neurons and microglia. In an AD mouse model, G protein-biased GPR3 signaling leads to decreased soluble Aβ levels and amyloid plaque area and, surprisingly, an increase in the area of microglia and astrocytes. Further transcriptomic analysis reveals enhanced activation of disease-associated-microglia (DAM) and neuroprotective A2 astrocytes in Gpr3HA-Ala mice and uncovers an astrocyte-microglia communication that modulates the neuroinflammatory response to Aβ accumulation.

Conclusions: To date, no GPCR has been shown to play a role in both the development of Aβ pathology and the innate immune reaction. Collectively, these studies strongly implicate GPR3-mediated β-arrestin signaling in neuronal regulation of Aβ accumulation and microglial and astrocytic regulation of neuroinflammation.
ON-DEMAND SYMPOSIUM: AD ANIMAL MODELS AND MECHANISTIC & TRANSLATIONAL ASPECTS 1

HUMAN APOA-I-MILANO VARIANT MOBILIZES SOLUBLE CEREBRAL BETA-AMYLOID AND IMPROVES COGNITION PERFORMANCE IN VERY OLD APP23 MICE

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Aims: Therapies based on apolipoprotein A-I (ApoA-I), classically tested for cardiovascular diseases, have been recently proposed for Alzheimer’s disease (AD). Based on a drug reprofiling approach, our objective was to explore the strategy of using a modified ApoA-I form, ApoA-I-Milano (M), as a treatment for this neurodegenerative disease. ApoA-I-M is a natural variant of APOA1 (containing the R173C mutation), which confers protection against atherosclerosis development.

Methods: In the present study, we treated intraperitoneally middle-aged and very old (12 and 21-month-old) APP23 mice for 8 weeks with human recombinant (r) ApoA-I-M protein or saline. Behavioral parameters as well as biochemical determinations were performed.

Results: In the middle-aged mouse group, rApoA-I-M treatment reduced the anxiety behavior associated with this AD model, whereas in very old mice, rApoA-I-M treatment reversed the alterations in T-maze performance, indicating a therapeutic impact on working memory. This cognitive improvement was accompanied by neuronal loss recovery in the polymorphic layer of the dentate gyrus. Although the subchronic treatment could not modify the insoluble levels of b-amyloid (Abeta), very old mice treated with rApoA-I-M showed lower brain Abeta40 soluble levels and elevated Abeta40 levels in cerebrospinal fluid (CSF). These results indicate the ability of rApoA-I-M to mobilize brain Ab from the periphery. Interestingly, vascular occludin expression was significantly increased in rApoA-I-M-treated brains, and plasma soluble RAGE was remarkably elevated in all rApoA-I-M-treated mice, which drastically decreased the AGEs/sRAGE ratio, a marker of endothelial damage.

Conclusions: rApoA-I-M offers therapeutic potential in the AD treatment through mechanisms involving Abeta mobilization and the restoration of cerebrovascular function.
Aims: Objectives: Paros Bio is developing an AAV gene therapy for the treatment of Autosomal Dominant Alzheimer's Disease (ADAD), an early onset form of AD (onset at <65 years of age). ADAD is mainly caused by mutations in the presenilin 1 (PSEN1) gene, which encodes the catalytic subunit of γ-secretase complex and is responsible for the cleavage of amyloid precursor protein (APP). This cleavage produces Aβ of varying lengths, with longer peptides like Aβ42 being more prone to aggregation than shorter peptides such as Aβ40. Of the >150 distinct, 100% penetrant PSEN1 mutations associated with ADAD, most result in a partial or nearly complete loss of γ-secretase function, resulting in decreased production of Aβ40 and an increase in the ratio of Aβ42:Aβ40. Aggregation of Aβ peptides into amyloid plaques in the brain is a hallmark of ADAD.

Methods: Paros Bio is employing a gene replacement approach to normalize γ-secretase function. Using an AAV vector, Paros has successfully expressed a functional copy of PSEN1 in in vivo and in vitro models of presenilin dysfunction.

Results: We have demonstrated that introduction of wildtype PSEN1 in mouse models and patient-derived cell lines effectively normalizes γ-secretase function. Our AAV vector can be broadly distributed in brain tissue and provides attractive expression of PSEN1.

Conclusions: Paros Bio has developed a vector that enables broad expression of WT PSEN1 and is capable of normalizing γ-secretase function in in vitro and in vivo models of ADAD.
SIRT2 REPRESSES SYNAPtic FRIZZLED RECEPTORS AT EARLY STAGES OF ALZHEIMER’S DISEASE

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Aims: The Wnt antagonist Dickkopf-1 is elevated in Alzheimer’s Disease (AD) and required for amyloid-β-mediated synapse loss. Nonetheless, the expression of other Wnt components remain unexplored in AD. The objective of this work is to analyse the mRNA levels and epigenetic regulation of Frizzled (Fzd) genes, which encode the main Wnt receptors, in early AD.

Methods: We evaluated 1) mRNA levels of 4 synaptic Fzds by qPCR in hippocampal samples from human AD and hAPPNL-G-F AD model, and 2) epigenetic changes by ChIP-qPCR. Next, we used pharmacological approaches to test the role of specific epigenetic regulators contributing to Fzds deregulation in AD.

Results: Our analyses demonstrate that Fzd1 and Fzd7 are down-regulated at early AD stages. We found reduced levels of the active histone mark H4K16ac at Fzd1 and Fzd7 promoters, accompanied by increased levels of Sirt2, a known H4K16ac deacetylase. In vitro and in vivo pharmacological inhibition of Sirt2 rescues H4K16c levels and expression of Fzd1 and Fzd7 in the hAPPNL-G-F model. In addition, we found that Sirt2 recruitment to Fzd1 and Fzd7 promoters depends on the activity of FoxO1, a transcription factor. Finally, we found reduced levels of inhibitory phospho-Sirt2 in nuclear samples and increased levels its phosphatase PP2C, leading to hyperactive nuclear Sirt2 and favouring Fzd1 and Fzd7 repression in AD.

Conclusions: Our results demonstrate that hyperactive nuclear Sirt2 downregulates Fzd1 and Fzd7 in early AD, via reducing H4K16ac levels at Fzd1 and Fzd7 promoters. These results postulate Sirt2 inhibition as a therapeutic target for boosting Wnt signalling and ameliorate AD.
Aims: Alzheimer’s disease (AD) is the leading cause of dementia worldwide. AD research field have mainly utilized mouse models for decades, but species differences between rodents and primates may constrain us from understanding the precise disease mechanisms.

Methods: We utilized Transcription Activator-Like Effector Nuclease (TALEN) and Base Editor to introduce familial AD-causing mutations into the presenilin 1-encoding gene (PSEN1) in the common marmoset (Callithrix jacchus), a small new world primate.

Results: We successfully deleted the 3’ SS of the PSEN1 gene (PSEN1-ΔE9) in the marmoset embryos by microinjection of TALEN, and obtained mutant PSEN1 neonates. Sequence analysis of mRNA in these embryos and neonates confirmed skipping of exon 9 as expected. Whole genome sequencing in the first mutant animal illustrated an inclusive absence of off-target effects. Quantitative analysis of amyloid-β (Aβ) in the cultured medium of primary fibroblasts showed elevation of Aβ42/Aβ40 ratio in PSEN1-ΔE9 fibroblasts. Western blot analysis detected uncleaved full-length PS1 protein as well as N- and C-terminal fragments of PS1 protein in the PSEN1-ΔE9 fibroblasts. In addition to PSEN1-ΔE9 marmosets, we recently succeeded to generate PSEN1-P117L marmosets using Base Editor.

Conclusions: To our knowledge, we have successfully generated the world’s first AD model of non-human primates. These animals are expected to show early amyloid pathology in the brain and to contribute to cutting-edge research to elucidate primate-specific mechanism in AD. In addition, we will make the mutant animals available to researchers worldwide in the fight against AD in the future.
ON-DEMAND SYMPOSIUM: AD ANIMAL MODELS AND MECHANISTIC & TRANSLATIONAL ASPECTS 1

NLRP3 MODULATES TAU PATHOLOGY AND NEURODEGENERATION IN TAU TRANSGENIC MICE

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Aims: Tauopathies are a family of neurodegenerative diseases characterized by the presence of aggregated tau, with Alzheimer’s disease (AD) as the most prevalent tauopathy. Tauopathies are furthermore characterized by neurodegeneration. We and others have previously demonstrated that NLRP3-ASC inflammasome is activated by pre-aggregated tau and contributes to tau pathology in non-seeded as well as in tau-seeded conditions. However, the modulatory role of NLRP3-inflammasome on neurodegeneration downstream of tau pathology has not been assessed.

Methods: We generated crosses of NLRP3 deficient mice (NLRP3-/-) with TauP301S transgenic mice (PS19; Tau) to obtain Tau.NLRP3-/- and Tau.NLRP3+/+ mice. In this model, we assessed tau pathology and hippocampal atrophy at 10 months of age. Moreover, by intracerebral injection of pre-aggregated tau-seeds, we assessed tau seeding and propagation as well as the associated hippocampal atrophy at the age of 8 months.

Results: Tau pathology in hippocampus and cortex was significantly decreased in Tau.NLRP3-/- mice at 10 months of age. Importantly, hippocampal area was significantly decreased in Tau.NLRP3+/+ versus Tau.NLRP3-/- mice. Following tau-seeding, tau pathology was significantly decreased both, in the ipsi- and contra-lateral, hippocampus and cortex in Tau.NLRP3-/- . Furthermore, hippocampal area was significantly decreased in the absence of NLRP3 in tau-seeded tau mice.

Conclusions: Our data further support a role of NLRP3, upstream of ASC, in tau pathology, in seeded and non-seeded conditions. We furthermore demonstrate a contributing role of NLRP3-inflammasome activation in tau-associated neurodegeneration in seeded and non-seeded conditions. These data support the importance of investigating the role of NLRP3-inflammasome as potential therapeutic target in tauopathies.
ON-DEMAND SYMPOSIUM: DISEASE MECHANISMS, INFLAMMATION. MICROGLIA, APOE ASTROCYTES, AMYLOIDS, LIPIDS, FATTY ACIDS

MONOMERIC C-REACTIVE PROTEIN VIA ENDOTHELIAL CD31 FOR NEUROVASCULAR INFLAMMATION IN AN APOE GENOTYPE DEPENDENT PATTERN: A RISK FACTOR FOR ALZHEIMER’S DISEASE?

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Aims: In chronic peripheral inflammation, endothelia in brain capillary beds could play a role for the apolipoprotein E4 (ApoE4)-mediated risk for Alzheimer’s disease (AD) development. It remains unknown whether peripheral monomeric CRP (mCRP) induces AD pathogenesis through some receptor of blood-facing endothelia in the brain in an ApoE genotype dependent fashion.

Methods: We used human samples, ApoE knock-in and deficient mouse models, and primary brain endothelia. Different ApoE mice were intraperitoneally (i.p.) injected with mCRP. The characterizations by immunostaining, proximity ligation assay (PLA) and siRNA were conducted to identify the receptor for mCRP. Brain microvessel and endothelia were isolated for RNA sequencing to explore the molecular pathway.

Results: Here we demonstrate that the interactions of endothelial CD31 with monomeric C-reactive protein (mCRP) vs. ApoE were linked with shortened neurovasculature for AD pathology and cognition. In ApoE knock-in mice, we discovered that intraperitoneal injection of mCRP, via binding to CD31 on endothelial surface and increased CD31 phosphorylation (pCD31), leading to cerebrovascular damage and the extravasation of T lymphocytes into the ApoE4 brain. While mCRP was bound to endothelial CD31 in a dose- and time-dependent manner, knockdown of CD31 significantly decreased mCRP binding and altered the expressions of vascular-inflammatory factors including vWF, NF-kB and p-eNOS. RNA-seq revealed endothelial pathways related to oxidative phosphorylation and AD pathogenesis were enhanced, but endothelial pathways involving in epigenetics and vasculogenesis were inhibited in ApoE4.

Conclusions: This is the first report providing some evidence on the ApoE4-mCRP-CD31 pathway for the cross-talk between peripheral inflammation and cerebrovasculature leading to AD risk.
Aims: We showed that poly-T repeat length in TOMM40 (TOMM40-523') is associated with AD risk in individuals carrying APOEɛ3 on European local ancestry (LA). ‘Very long’ repeats (VL, >29T) have a protective effect compared to ‘short’ repeats (S, <19T). We hypothesized that variants in linkage disequilibrium (LD) with TOMM40-523’ on the European LA APOEɛ3 haplotype can modify risk for AD, potentially through APOE regulation.

Methods: We used the short tandem repeat detection bioinformatics algorithm HipSTR to type S and VL repeats in whole genome sequencing data of individuals homozygous for the APOEɛ3 European LA haplotype from the Puerto Rico AD Initiative project. Frequency of variants on 16S and 14 VL haplotypes were compared to determine LD with the repeat. HaploView was used to determine the LD structure of the repeat and surrounding variants.

Results: We identified a 16kb LD block surrounding TOMM40-523’ harboring 21 variants in strong LD ($r^2 > 0.9$) with the repeat (hg19, chr19:45,395-45,411k); including the APOE promoter and several potential enhancer regions. Assessment of combined regulatory activity of variants in LD with the repeat on S or VL haplotypes in these regions is currently ongoing using luciferase reporter assays in AD-relevant cell types (i.e. astrocytes, microglia and neurons).

Conclusions: The identification of distinct S and VL haplotypes on APOEɛ3 European LA background in promoter and close-by enhancers support importance of the surrounding variants in the risk effect observed in the association analyses. The follow-up functional data will pinpoint the regulatory TOMM40-523’ LD variant(s) driving the signal for the observed different AD risk effects in European ancestry APOEɛ3 carriers.
ON-DEMAND SYMPOSIUM: DISEASE MECHANISMS, INFLAMMATION. MICROGLIA, APOE ASTROCYTES, AMYLOIDS, LIPIDS, FATTY ACIDS 1

MYO-INOSITOL CONCENTRATION REFLECT THE EFFECT OF APOE E4 ALLELE ON THE ASSOCIATION BETWEEN ASTROCYTES FUNCTION AND AMYLOID-BETA

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Aims: A growing body of evidence suggests that astrocytes play a major role in the pathophysiology of Alzheimer’s disease (AD). Astrocytic function could also be a gateway of APOE-ε4 to AD pathology, considering that APOE is primarily expressed in astrocytes. Myo-Inositol is a metabolite involved in several critical astrocytic functions and can be quantified in-vivo with magnetic resonance spectroscopy (MRS). In the present study we explored the interaction between APOE, astrocytic function and protein aggregation focusing on cognitively unimpaired individuals and prodromal AD patients.

Methods: We investigated the association between myo-Inositol (mIns) concentration, as quantified with MRS, and both Aβ and tau accumulation as quantified by ¹⁸F-flutemetamol and, ¹⁸F-RO948 PET respectively. We also tested the possible impact of APOE on such associations. The study included healthy elderly and patients with mild cognitive impairments from the Swedish BioFINDER-2 cohort (N=428) and the MRS analysis was performed focusing on the precuneus area. To further investigated the involvement of astrocytes in the processes under exam, we also quantified plasma glial fibrillary acidic protein (GFAP), in a subgroup of participant with available GFAP data (N=288).

Results: The analysis revealed that myo-Inositol correlated primarily with Aβ and only in APOE-ε4 carriers (figure 1A-B). In addition to that, the analysis of GFAP plasma data showed an association of Aβ-SUVR with GFAP levels but no interaction between APOE and GFAP levels (figure 1C).
Conclusions: The current results suggest that the quantification of mIns levels with MRS could provide a preferential marker of the impact of APOE-ε4 allele on Aβ accumulation.

Figure 1. A: Effect of moderation of APOE on the association between mIns and Aβ (mIns*APOE: β=1.21, p<0.001). B: voxel-wise regression of Aβ-SUVR maps against mIns in the precuneus region. The heatmap showed the cluster of significant associations (FWE, p<0.05). C: Association between plasma GFAP levels and Aβ-SUVR values stratified by APOE genotype. No moderation effect of APOE on the association between plasma GFAP and Aβ was found (GFAP*APOE, β=0.0005 p>0.1).

Conclusions: The current results suggest that the quantification of mIns levels with MRS could provide a preferential marker of the impact of APOE-ε4 allele on Aβ accumulation.
ON-DEMAND SYMPOSIUM: DISEASE MECHANISMS, INFLAMMATION. MICROGLIA, APOE ASTROCYTES, AMYLOIDS, LIPIDS, FATTY ACIDS

BRAIN LIPID CHANGES PRECEDE AND INDUCE PROTEINOPATHY AND NEUROINFLAMMATION

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Aims: Neurons and glia depend on tight regulation and exchange of lipids for proper function. Recent work shows that lipid disturbances that develop over time and with age including altered mechanisms of glia-neuron exchange of lipids and inflammatory signals, create neuropathology in brain regions vulnerable to neurodegeneration.

Methods: In order to investigate the interplay between neurons, astrocytes and microglia, with altered lipid content and trafficking, we tested (1) the effects of increased glycolipid formation by deficiency of glucocerebrosidase (GBA) or β-hexosaminidase (HEX) lysosomal enzymes, or (2) ApoE function including cholesterol transport. (3) We explored in vivo the cellular and brain changes that occur in the aging brain by examining long-term changes in lipid distribution and its relationship to α-synuclein, tau, other structural and synaptic proteins.

Results: The experiments demonstrate that (a) lysosomal enzymes decrease in expression and function with age, simulating haploinsufficiency in rare genetic brain disease. (b) in Parkinson’s disease patients and in induced deficiencies in animal models of lysosomal enzymes, there is a redistribution of intracellular lipids, that can be rescued by lysosomal gene therapy gain of function. (c) neuroinflammatory cascades are related to the lipid changes, and are associated with microglial activation. (d) the α-synucleinopathies are subsequent to the lipid accumulation, and are reversed by lipid reversing gene therapy.

Conclusions: These results show how lipid dyshomeostasis, changes in lipid transport and clearance precede proteinopathies. Such mechanisms are therefore central causative factors in neurodegeneration. New therapies that correct pathogenic cellular lipid disturbances could potentially prevent disease progression in Parkinson’s disease and dementia.
ON-DEMAND SYMPOSIUM: DISEASE MECHANISMS, INFLAMMATION. MICROGLIA, APOE ASTROCYTES, AMYLOIDS, LIPIDS, FATTY ACIDS

IS TOXIC AMYLOID FORMATION TRIGGERED BY PEPTIDE LIPIDATION?

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Aims: Many challenges remain in understanding the link between fibrillogenesis and biological outcomes as it is frequently difficult to relate the in vitro behaviour to the in vivo toxicity. However, it is generally recognised that lipid membranes play a direct role in promoting amyloid formation. It is hypothesised that lipidation of peptides such as Abeta by direct acyl transfer from membrane lipids provides a route for driving their nucleation (doi: 10.1002/bies.201900147).

Methods: The lipidation of peptides, protein and small organic molecules has been studied in model liposome systems by liquid chromatography - mass spectrometry (LC-MS).

Results: Some peptides are readily lipidated in liposomes to form a lipidated peptide alongside a lysolipid byproduct. In short unstructured peptides, this lipidation triggers the adoption of secondary structure. Peptide lipidation is sequence and lipid dependent. Some molecules, most notably drugs, have also been found to be lipidated in vivo.

Conclusions: Lipidation appears to be a general property of molecules embedded in lipid membranes. This route for triggering amyloid formation in vivo is appealing because it is able to account for a number of disparate phenomena concerning amyloid formation, including: the dependency of amyloid formation on cholesterol levels; the nucleation of fibre formation in response to changes in the lipidome, such as may occur during oxidative stress or disease states; the formation of amyloid deposits at sites distal to peptide generation; and the effects of sequence variation on amyloid formation. The lipidation hypothesis is additionally appealing because lipidated amyloid peptides will be intrinsically lipophilic and thereby tagged to the membrane.

![Diagram of APP and Aβ processing](image-url)
ON-DEMAND SYMPOSIUM: DISEASE MECHANISMS, INFLAMMATION. MICROGLIA, APOE
ASTROCYTES, AMYLOIDS, LIPIDS, FATTY ACIDS 1

EQUILIBRATING FATTY ACID HOMEOSTASIS FOR PARKINSON'S DISEASE THERAPEUTICS

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Aims: Although the PD-causing protein α-synuclein (αS) interacts with lipids and fatty acids (FA) physiologically and pathologically, targeting FA homeostasis for therapeutics is in its infancy. We identified the PD-relevant target stearoyl-coA desaturase (SCD): inhibiting monounsaturated FA synthesis reversed PD phenotypes. An SCD inhibitor is now in human clinical trials. The Aim for our current work was to establish whether modifying other lipid pathway components could equilibrate lipid/FA dyshomeostasis in PD patient-derived neurons. We sought to develop a unique therapeutic strategy for patients over long-term treatment.

Methods: Primary models included human neuroblastoma cells, human neurons expressing the familial PD αS mutation E46K and patient-derived αS triplication neurons with isogenic corrected controls. Genetic and biochemical dissections were performed to assess how FA generation in lipid pathways could be pursued as novel therapeutic targets. In vivo analysis was performed in an αS-induced dopaminergic neurodegeneration C.elegans model. Comprehensive FA and lipid profiling provided mechanistic insight into the basis of rescue by a new candidate therapeutic target.

Results: We determined alterations in the neutral lipid pathway as a target for PD therapeutics. We identified two inhibitors as rescuing disease-relevant phenotypes including the abnormal increase in phosphorylated αS and decreased αS tetramer:monomer ratio associated with triplication of the αS locus. Equilibrizing cellular FAs reduced αS inclusion formation, decreased the aberrant unfolded protein response associated with αS triplication patient-derived neurons and alleviated αS-induced dopaminergic neurodegeneration in C.elegans.

Conclusions: New targets that re-establish cellular FA and lipid homeostasis are promising targets for PD therapeutics.
ON-DEMAND SYMPOSIUM: DISEASE MECHANISMS, INFLAMMATION. MICROGLIA, APOE ASTROCYTES, AMYLOIDS, LIPIDS, FATTY ACIDS

HUMAN NEURAL CELL TYPE-SPECIFIC EXTRACELLULAR VESICLE PROTEOME DEFINES DISEASE-RELATED MOLECULES ASSOCIATED WITH ACTIVATED ASTROCYTES IN ALZHEIMER’S DISEASE BRAIN

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Aims: Extracellular vesicles (EVs) are known to transfer pathogenic molecules in Alzheimer’s disease (AD) in a cell-type specific manner. Transcriptomic or proteomic profiling of cell type-specific EVs from patient-derived biopsies is helpful to study the pathophysiology of AD. Our study seeks to define human CNS cell type-specific EV protein signatures that could be employed for cell type EV isolation, and investigate their potential roles in AD pathology.

Methods: Label-free and tandem mass tag-labeling based quantitative mass-spectrometry of EVs isolated from human induced pluripotent stem cells (hiPSCs) and AD brain tissues were conducted. AD-associated and cell type-specific EV protein modules were generated from the weighted protein co-expression network analysis (WGCNA). The significance of disease-related proteins was further validated by purifying cell type EVs from AD brain using an independent cohort.

Results: Novel cell type-specific EV protein markers were identified from hiPSC-derived excitatory neurons (e.g., NCAM1, ATP1A3), astrocytes (e.g., LRP1, ITGA6), microglia-like cells (e.g., CD300A, LCP1) and oligodendrocytes (e.g., LAMP2, FTH1). Furthermore, WGCNA of brain-derived EV proteomics from 11 healthy controls, 8 mild cognitive impairment and 11 AD patients identified a protein module, which was enriched in astrocyte-derived EV markers and most significantly associated with AD pathology and cognitive function. The hub protein from this module, integrin-β1 (ITGB1), was significantly elevated in astrocyte-specific EVs enriched from total brain-derived AD EVs and associated with brain b-amyloid and tau load in independent cohorts.

Conclusions: Our study provides a featured framework and rich resource for analyses of EV functions in neurodegenerative diseases in cell type-specific manner.
ON-DEMAND SYMPOSIUM: DISEASE MECHANISMS, INFLAMMATION. MICROGLIA, APOE ASTROCYTES, AMYLOID, LIPIDS, FATTY ACIDS

CELL-WIDE CALCIUM DYSFUNCTION IN ASTROCYTES THROUGHOUT CORTEX OF AWAKE APP/PS1 MICE

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Aims: Astrocytes are predominant glial cells that exhibit spontaneous intra- and intercellular calcium dynamics that strongly modulate synaptic activity, vascular dynamics, neurovascular coupling and sleep, which are all affected by Alzheimer’s disease (AD). Advancing our understanding towards pharmacological protection and/or restoration of astrocytes is an exciting research avenue towards astrocyte-targeting therapies. We tested the hypothesis that amyloid plaques disrupt all spontaneous calcium dynamics throughout all cellular compartments of astrocytes (somata, primary processes, fine processes and endfeet) in the awake mouse brain.

Methods: APP/PS1 mice and age-matched non-transgenic littermates (12-17 months old; n=10) were anesthetized during surgical intracortical injection of our genetically encoded calcium indicator, gfa2.yc3.60, into both hemispheres of the somatosensory cortex followed by ~ 6 mm cranial window over both injection sites and a head-post secured to the skull to permit habituation to awake multiphoton imaging. Cortical volumes containing astrocytes and high magnification time-lapses at 2.3 Hz were acquired. Custom-written MATLAB scripts were used to quantify all spontaneous intra- and intercellular calcium dynamics in awake mice.

Results: Cortical astrocytes have cell-wide elevated resting calcium both near and far from plaques. Astrocytic intracellular calcium events are compartmentalized within processes and pathologically impacted by amyloid plaques. Spontaneous intercellular calcium events involve endfeet and propagate at ~33 microns/second throughout the cortex of awake mice. Spontaneous calcium events occurred with greater amplitude within somata.

Conclusions: Astrocytic spontaneous intra- and intercellular calcium events are heterogeneous and dysfunctional throughout all cellular compartments (somata, primary processes, fine processes and endfeet). Astrocyte-targeting therapeutic strategies should consider compartmentalization and heterogenous cell-wide dysfunction.
ON-DEMAND SYMPOSIUM: DISEASE MECHANISMS, INFLAMMATION. MICROGLIA, APOE ASTROCYTES, AMYLOIDS, LIPIDS, FATTY ACIDS 1

REDUCTION OF E3 LIGASE IDOL ENHANCES MICROGLIAL ACTIVATION AND PHAGOCYTOSIS OF FIBRILLAR AB

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Aims: Brain lipoprotein receptors regulate the metabolism and signaling of ApoE and are potential therapeutic targets for AD. Previously, we discovered that E3 ubiquitin ligase IDOL is a negative regulator of brain lipoprotein receptors, and genetic deletion of IDOL decreases Aβ levels in APP/PS1 mice. In this study, we aim to define therapeutic benefits of reducing brain IDOL levels in mouse models of β-amyloidosis, and to investigate the underlying mechanisms of action.

Methods: We utilized an antisense oligonucleotide (ASO) to reduce IDOL expression in the brains of APP/PS1 and AppNL-GF mice, and performed single cell RNA-seq to profile the changes of transcriptome in brain cells. To evaluate microglial phagocytosis of Aβ in vivo, we labeled fibrillar Aβ (fAβ) with fluorescent dye methoxy-X04 and quantified X04(+) microglia in dissociated brain cells using flow cytometry. Finally, we evaluated alterations in Aβ pathology and cognitive functions after chronic reduction of IDOL.

Results: IDOL reduction upregulated lysosomal/phagocytic genes in microglia, and increased subpopulation of disease-associated microglia (DAM). Consistent with transcriptome profile, acute IDOL reduction markedly increased percentage of microglia containing fAβ, and increased protein levels of TREM2, a well-known DAM marker, in microglia associated with Aβ plaque. Chronic IDOL reduction slowed the progression of Aβ pathology, and improved spatial learning and memory.

Conclusions: Our results suggested that reducing IDOL levels in the brain facilitates microglial activation, promotes phagocytic clearance of Aβ, and ameliorates Aβ pathology. Pharmacological inhibition of IDOL activity in the brain may represent a novel therapeutic strategy for AD treatment.
Aims: ApoE gene is implicated in different aspects of neurogenesis although its detailed molecular mechanisms are not fully understood.

Methods: To better understand the regulatory roles of ApoE in neurogenesis, we differentiated isogenic control and ApoE−/− human neuronal stem cells (NSC) into mature cortical neurons and cultured them for additional three weeks.

Results: Like control NSC, ApoE−/− NSC differentiates into mature neurons, indicating that ApoE gene is not required for differentiation. However, prolonged culture of mature ApoE−/− neurons led to proliferation of fibroblast-like cells with increased ROS level and DNA damage responses. Interestingly, ApoE is only expressed in control NSC and neuronal progenitor cell (NPC) stages, but not in mature neurons, suggesting that ApoE may epigenetically regulate the integrity and maintenance of the mature neurons. Mechanistic studies revealed that absence of ApoE in NSC led to upregulation of several microRNAs, including miR-1301-3p. These microRNAs targeted EZH1 to reduce its protein expression, resulting in the lower level of repressive H3K27me3 marks in ApoE−/− NPC and mature neurons. Chromatin immunoprecipitation experiment suggests that the lower level of H3K27me3 at many extracellular matrix organization and angiogenesis genes is responsible for their higher expression and aberrant phenotypes observed in ApoE−/− neurons.

Conclusions: Taken together, our results indicate that ApoE maintains pure population of mature neurons via H3K27me3-mediated repression of genes that drive fibroblast proliferation in NPC stage.
ON-DEMAND SYMPOSIUM: DISEASE MECHANISMS, INFLAMMATION. MICROGLIA, APOE ASTROCYTSES, AMYLOIDS, LIPIDS, FATTY ACIDS

THE R522 PROTECTIVE VARIANT IN PLCG2 APPEARS TO PROTECT AGAINST ALZHEIMER'S DISEASE DEVELOPMENT BY REDUCING NEUROINFLAMMATION AND CORTICAL PLAQUE BURDEN

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Aims: Evidence from GWAS suggests the R522 variant in the enzyme PLCG2 protects against Alzheimer's disease (AD). Within cells, PLCG2 breaks down the phospholipid PI(4,5)P2, releasing IP3 and DAG and activating various signalling pathways. PLCG2 lies downstream of TREM2, overexpression of which is known to slow AD development by inhibiting amyloid plaque formation. In the brain, PLCG2 is predominantly expressed in microglia. We aimed to characterize how the R522 mutation affects both PLCG2 enzyme activity and subsequent microglial function.

Methods: We studied the effects of the R522 mutation in novel human and mouse models, characterising PLCG2 activity in microglia and macrophages by measuring Ca2+ and PI(4,5)P2 in the presence of a PLCG2 activator. Amyloid uptake, cytokine secretion, TREM2 function, and plaque burden were examined in cells and mice to characterise phenotypic changes.

Results: R522 exhibited a hyper-functional PLCG2-mediated Ca2+ response, with this hyper-function appearing to result in a reduced PI(4,5)P2 pool. In addition, R522 models showed attenuated LPS-mediated cytokine production, increased TREM2 signalling, enhanced amyloid uptake and reduced cortical plaque burden.

Conclusions: That these findings were largely consistent across physiologically relevant human and mouse models provides compelling evidence of increased enzyme activity in R522 microglia and macrophages, explaining reduced PIP2 levels seen in mice. This PLCγ2 substrate depletion could prevent chronic enzyme activation and explain the attenuated inflammatory cytokine response following LPS-activation. Moreover, upregulated TREM2 signalling appears to enhance amyloid uptake and reduce cortical plaque burden. Together, these phenotypic changes could help explain the protective effects of R522 in patients.
Increased Protein Kinase C Activity in Human Brain Microvascular Endothelial Cells in Response to Apolipoprotein E4 Isoform

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Aims: Apolipoprotein E (apoE) is a genetic risk factor for the development of Alzheimer’s disease and sporadic cerebral amyloid angiopathy. The apoE4 isoform enhances the risk of intracerebral hemorrhages by reduced amyloid-β clearance and increased protein accumulation in cerebral vessel walls. Protein kinase C (PKC) is one of the kinases regulating signal transduction in these vascular endothelial cells of the blood-brain barrier (BBB). We aimed to investigate the effect of different apoE isoforms on PKC activity in the context of BBB integrity.

Methods: Immortalized human cerebral microvascular endothelial cells were cultured to confluence. After treatment with lipidated apoE3 or apoE4 for 6/24/48 hours, cells were lysed and analyzed with SDS-PAGE and Western blotting using an antibody detecting the phosphorylated PKC substrate motif. Putative PKC substrates were isolated by immunoprecipitation and analyzed via mass spectrometry for protein identification.

Results: Western blotting of lysates revealed a remarkable concentration-dependent increase of a band with a molecular weight of 250kDa after six hours of apoE4 treatment as compared to apoE3. Combined immunoprecipitation and mass spectrometry analysis yielded multiple identifiable proteins, five of which were high molecular weight proteins containing phosphorylated sequences highly homologous to the predicted PKC motif. Mass spectrometry analysis with the aim to identify the exact nature of the apoE4-mediated phosphorylation of PKC substrates is ongoing.

Conclusions: The increased phosphorylation of PKC substrates indicates an amplification of PKC activity induced by apoE4 presence. Identification of these phosphorylated PKC substrates in cerebrovascular cells may help to unravel the mechanisms of apoE4-mediated BBB dysfunction.
ON-DEMAND SYMPOSIUM: DISEASE MECHANISMS, INFLAMMATION. MICROGLIA, APOE ASTROCYTES, AMYLOIDS, LIPIDS, FATTY ACIDS 1

MEMBRANE REPAIR IN ALZHEIMER'S DISEASE

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Aims: Alzheimer's disease is characterized by amyloid plaques surrounded by dystrophic neurites full of stalled vesicles and lacking synaptic function. Dystrophic neurites accumulate amyloid precursor protein, and β- and γ-secretases, leading to further Aβ production. We aim to understand cellular mechanisms of dystrophic neurite formation, and to prevent or correct them. We hypothesize that Aβ causes membrane disruption, calcium dyshomeostasis and breakdown of microtubule transport and synaptic function. We investigate the potential of annexin A6 and other membrane repair proteins to prevent dystrophic neurite formation in AD.

Methods: Cultured neurons from wild type mice and mice expressing A6-tGFP from the genomic locus were laser-injured, then imaged with multiphoton microscopy. Parallel cultures were fixed and stained for immunofluorescence or lysed to be used in immunoblot analysis. A6-tGFP mice were crossed to 5XFAD mice, and offspring were analyzed at 4-6 months of age by immunoblot and immunofluorescence. Overexpression of A6-GFP was induced by intracerebroventricular injection of P0 mouse pups which were harvested at 4 months old for analysis by immunoblot and immunofluorescence.

Results: A6-tGFP localizes to membrane damage in injured neurons. In the brain, A6-tGFP and endogenous A6 show membrane localization in neurons, and at the membrane of plaque-associated dystrophic neurites, especially those with a high level of Aβ. AAV-mediated overexpression of A6-GFP results in A6-GFP in more neurons and dystrophic neurites.

Conclusions: A6 localizes to site of membrane injury in neurons, and to dystrophic neurites near plaques in the brain, indicating it may play a role in repairing amyloid mediate membrane damage.
ON-DEMAND SYMPOSIUM: GENETICS OF NEURODEGENERATION 3

MULTI-TISSUE PROTEOMICS IDENTIFIES MOLECULAR SIGNATURES FOR SPORADIC AND GENETICALLY DEFINED ALZHEIMER DISEASE CASES


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Aims: Alzheimer disease (AD) is a heterogeneous disease with many associated genes. Multi-tissue proteomic signatures for sporadic and genetically defined AD (e.g., pathogenic variant carriers in APP and PSEN1/2 and risk variant carriers in TREM2) will illuminate the biology of this heterogeneous disease.

Methods: We present one of the largest multi-tissue proteomic profiles based on 1,305 proteins in brain (n=360), cerebrospinal fluid (CSF; n=717), and plasma (n=490) from the Knight Alzheimer Disease Research Center (Knight ADRC) and Dominantly Inherited Alzheimer Network (DIAN) cohorts.

Results: We identified proteomic signatures for sporadic AD status and replicated them in multiple, independent datasets. The area under the curve (AUC) for CSF proteins was 0.89 and 0.90 in discovery and replication data, respectively, significantly higher than the AUC for CSF p-tau181/Aβ42 (AUC=0.81; P=2.4×10-6). We also identified a proteomic signature that differentiated TREM2 variant carriers from sporadic AD cases and controls with high sensitivity and specificity (AUC=0.81-1). Several proteins associated with autosomal dominant AD (ADAD) in brain also replicated in CSF. The proteins associated with sporadic AD status were also altered in ADAD, but with greater effect size (1.4 times, P=3.8×10-5). Enrichment analyses highlighted several pathways including AD (calcineurin, APOE, GRN), Parkinson disease (α-synuclein, LRRK2), and innate immune response (SHC1, MAPK3, SPP1).

Conclusions: Our findings show the power of multi-tissue proteomics’ contribution to the understanding of AD biology and to the creation of tissue-specific prediction models for individuals with specific genetic profiles, ultimately supporting its utility in creating individualized disease risk evaluation and treatment.
ON-DEMAND SYMPOSIUM: GENETICS OF NEURODEGENERATION 3

THE GLOBAL PARKINSON'S GENETICS PROGRAM (GP2) - PROGRESS SO FAR

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Aims: The Global Parkinson’s Genetics Program (GP2, http://gp2.org/) aims to dramatically expand the current understanding of the genetic architecture of Parkinson’s disease (PD) and to make that understanding globally relevant.

Methods: GP2 has created a structure to enable large scale data collection, production, analysis, and dissemination. Three main scientific outcomes are prioritized 1) enabling a dramatic expansion of genetic contributors 2) accelerating and improving genetic discovery in monogenic disease and 3) making these findings globally relevant. To achieve these outcomes, GP2 has the following structural priorities 1) Diversity in Research and Researchers 2) Democratization of Data 3) Collaboration and Cooperation 4) Safe, Responsible Data Sharing 5) Transparency & Reproducibility and 6) Foundational, Actionable Resource Production.

Results: We have created a steering committee and working groups (WGs). These WGs have established standards and review criteria for sample/cohort inclusion, compliance for data and sample sharing, and policies relating to authorship and sharing. A partnership was created to allow data sharing, complying with varied local regulations. We have also introduced global training programs.

Conclusions: GP2 aims to accelerate the next wave of discovery, and to make this work globally relevant, emphasizing research in underrepresented populations and enabling underrepresented researchers to drive this work forward. We believe that the findings and data generated from GP2 including new genetic associations, relationships between mutations, protective variants, commonalities, and differences in the genetics of disease in individuals of diverse ancestry can help clinicians, investigators and companies better understand who may develop PD, at what time, and to what degree.
ON-DEMAND SYMPOSIUM: GENETICS OF NEURODEGENERATION 3

RARE PSAP VARIANTS AND POSSIBLE INTERACTION WITH GBA IN REM SLEEP BEHAVIOR DISORDER


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Aims: PSAP encodes saposin C, the co-activator of glucocerebrosidase, encoded by GBA. Since GBA mutations are associated with idiopathic/isolated REM sleep behavior disorder (iRBD), a prodromal stage of synucleinopathy, we examined the role of PSAP mutations in iRBD.

Methods: We fully sequenced PSAP and performed Optimized Sequence Kernel Association Test in 1,113 iRBD patients and 2,324 controls.

Results: We identified loss-of-function (LoF) mutations, which are very rare in PSAP, in three iRBD patients and none in controls (uncorrected p=0.018). Two variants were stop mutations, p.Gln260Ter and p.Glu166Ter, and one was an in-frame deletion, p.332_333del. All three mutations have a deleterious effect on saposin C, based on in silico analysis. In addition, the two carriers of p.Glu166Ter and p.332_333del mutations also carried a GBA variant, p.Arg349Ter and p.Glu326Lys, respectively. The co-occurrence of these extremely rare PSAP LoF mutations in two (0.2%) GBA variant carriers in the iRBD cohort, is unlikely to occur by chance (estimated co-occurrence in the general population based on gnomAD data is 0.00035%). Although none of the three iRBD patients with PSAP LoF mutations have phenocverted to an overt synucleinopathy at their last follow-up, all manifested initial signs suggestive of motor dysfunction, two were diagnosed with mild cognitive impairment and all showed prodromal clinical markers other than RBD. Their probability of prodromal PD, according to the Movement Disorder
Society research criteria was 98% or more.

**Conclusions:** These results suggest a possible role of PSAP variants in iRBD and potential genetic interaction with GBA, which requires additional studies.
GWAS OF GENETIC RESILIENCE TO AGE-RELATED RISK FOR ALZHEIMER’S DISEASE IN AFRICAN-AMERICANS

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Aims: Identify whether genetic variants can confer resilience to age-related Alzheimer’s disease (AD) risk in admixed African-Americans.

Methods: Participants were ages 60+, of African ancestry (>=25%), and diagnosed as cases or controls. Genetic data were available from whole-genome-sequencing (WGS) or SNP arrays imputed to TOPMed. Genome-wide association studies (GWAS) were performed per data type, split by Hispanic/non-Hispanic participants (Table-1), followed by meta-analysis (Plink v2.0; GWAMA v2.2.2). GWAS performed multiple linear regression on an AD-age score that models resilience to age-related risk for AD (Le Guen & Belloy et al. 2021; Figure-1). Models adjusted for sex, APOE*4/APOE*2 dosage, the first five genetic principal components, and array/sequencing center.

Results: We found a novel protective genome-wide significant intergenic variant (rs77450754) ~200kb downstream of ATXN8OS/KLHL1 (Figure-2-3). It has a MAF of 12% in African ancestry samples, but is rare (0.1%) in Europeans.
<table>
<thead>
<tr>
<th>African-American Samples</th>
<th>Participants (N)</th>
<th>Cases (N (%))</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOPMed-Imputed – non-Hispanic</td>
<td>2,508</td>
<td>317 (12.6%)</td>
</tr>
<tr>
<td>WGS – non-Hispanic</td>
<td>2,683</td>
<td>1,060 (39.5%)</td>
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<tr>
<td>TOPMed-Imputed – Hispanic</td>
<td>1,067</td>
<td>556 (52.1%)</td>
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<tr>
<td>WGS – Hispanic</td>
<td>1,012</td>
<td>401 (39.6%)</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>7,270</strong></td>
<td><strong>2,334 (32.1%)</strong></td>
</tr>
</tbody>
</table>

Table-1. Sample demographics.

Figure-2. Manhattan plot. Green and red dots respectively indicate suggestive and genome-wide significance. Top variants are annotated with nearest gene.
Figure 3. Top hit rs77450754. A) Forest plot. B) Locus zoom plot.

Conclusions: Our results provide new insights into genetic resilience to AD across aging and emphasize the importance of including ancestrally-diverse populations in genetic studies.
ON-DEMAND SYMPOSIUM: GENETICS OF NEURODEGENERATION 3

MED13 IS A GENETIC MODIFIER OF SNCA

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Aims: To identify genetic modifiers of α-synuclein through a genetic screen in Drosophila, and to investigate mechanisms by which modifiers influence neurodegeneration.

Methods: We used transgenic Drosophila that expressed human α-synuclein to screen for dominant genetic modifiers from 3500 chemically mutagenized fly strains using a combination of locomotor assays and brain morphological analyses.

Results: From the screen, we identified 12 genetic modifiers of α-synuclein; all 12 have human orthologs and all but one are disease-associated. We further investigated the role of Med13 because its human ortholog lies close to a Parkinson’s disease GWAS risk locus, rs11658976. In the brains of flies and mice expressing human α-synuclein, Med13, Hypoxia Inducible Factor (HIF) and a few glycolytic enzymes were upregulated. Med13 is a component of the kinase module of the Mediator complex that bridges transcription factors bound at enhancer elements with the transcriptional machinery at the promoters. HIF is known to upregulate enzymes encoding glycolytic enzymes in a manner that requires the Med13-containing kinase module. Downregulation of Med13 and HIF in flies did not cause neurodegeneration, but when combined with α-synuclein expression, these manipulations enhanced α-synuclein-associated neurodegeneration. A combination of Med13 knockdown and α-synuclein expression in flies strongly downregulated glycolytic enzymes, indicating that Med13 is required for the upregulation of glycolytic enzymes in neurons stressed by synuclein toxicity.

Conclusions: These data suggest that Med13 is a strong candidate for the risk gene near rs11658976 for Parkinson’s disease, and that upregulation of glycolysis may be a viable therapeutic strategy for Parkinson’s disease.
ON-DEMAND SYMPOSIUM: GENETICS OF NEURODEGENERATION 3

LATENT FACTOR MODEL DETECTS AXIS OF DISEASE PROGRESSION ASSOCIATED WITH INCREASED LONGITUDINAL DECLINE OF GENETIC VARIANT SUBJECTS IN PD

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Aims: The absence of specific and objective metrics of disease progression in Parkinson’s Disease (PD) poses a major obstacle to early and accurate diagnosis and treatment. In this study we therefore utilized a novel latent variable model (LVM) to separate out different drivers of variability shared across multiple scores and assessments, and evaluated unique longitudinal trajectories associated with these factors and genetic variants.

Methods: We employed LVM techniques that explain covariation across multiple data types, allowing for improved signal detection. This approach was applied to clinical assessments, fluid biomarker levels, and transcriptomic measurements from the PPMI cohort to infer patient-specific latent disease trajectories. We then ran post-hoc linear mixed effects models on the LVM outputs to explore within and between subject longitudinal trajectories.

Results: Our model identified three latent factors, with the third relating to symptoms associated with more severe disease state (i.e. akinetic/rigid symptoms, worse cognitive function, lower striatal binding ratios, and higher levels on fluid biomarkers). Post-hoc analyses revealed that subjects with LRRK2 and GBA variants had higher scores on the third factor overall, while subjects with the LRRK2 variant also had increased rates of decline along this factor over time, relative to PD subjects with no genetic variants.

Conclusions: Our LVM model detected a latent axes of disease progression associated with more severe PD symptoms that declined at a quicker rate for subjects with the LRRK2 variant. This methodology and findings may therefore ultimately aid in the prognosis and treatment of individual PD patients.
ABERRANT EXON 2 SKIPPING DECREASES ATP6AP2 DOSAGE AFFECTING NEURAL DEVELOPMENT NETWORKS IN PATIENTS WITH X-LINKED INTELLECTUAL DISABILITY, EPILEPSY AND PARKINSONISM

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Aims: Mutations that alter splicing of X-linked ATP6AP2 cause a spectrum of neurodevelopmental and neurodegenerative pathologies in affected males. Previously reported splicing mutations increased the level of a minor isoform with skipped exon 4 (de4) that encoded a functionally deficient protein. We aimed to measure de4 level in the brains of carriers of c.345C>T splicing mutation investigate a pathogenic mechanism of c.168+6T>A reported in a family with X-linked intellectual disability, epilepsy and Parkinsonism.

Methods: Nanostring nCounter of RNA from brain sections was used for gene expression profiling with custom-made probes to exon-exon junctions in ATP6AP2 We generated inducible pluripotent cells from patients with c.168+6T>A and re-programmed them to neural progenitor cells (NPCs). We performed RNA-seq and pathway analyses of NPCs and whole blood of carriers of c.168+6T>A.

Results: We demonstrated that de4 became the main ATP6AP2 transcript in the brains of carriers of c.345C>T We demonstrated that a c.168+6T>A increased skipping of exon 2; the resulting out-of-frame splicing introduced premature termination codons causing 50% reduction of ATP6AP2 expression. NPCs derived from the patients exhibited downregulated neural development gene networks. Analysis of peripheral blood transcriptomes of c.168+6T>A carriers identified several potential biomarkers of the ATP6AP2 deficiency in non-neural tissues.

Conclusions: Alternative splicing of de4 isoform is highly augmented in the human brain, as compared to non-CNS tissues. It is further enhanced by c.345C>T that may explain CNS-restricted clinical presentations in the patients. c.168+6T>A revealed a distinct defect of splicing. The resulting decrease of ATP6AP2 dosage is responsible for a neurodevelopmental disorder with early onset Parkinsonism.
A NOVEL SEX-SPECIFIC KEY DRIVER GENE OF ALZHEIMER'S DISEASE


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Aims: Alzheimer's disease (AD) is a progressive and age-associated neurodegenerative disorder that affects women disproportionately. However, the underlying mechanisms are poorly characterized. The aims of our studies are to identify sex-specific molecular networks and key driver genes of AD.

Methods: We integrated large-scale human postmortem RNA expression datasets from two human brain cohorts (MSBB and ROSMAP) to perform multiscale network analysis and identify key drivers with sexually dimorphic expression patterns or differentially respond to APOE genotypes between sexes. The expression patterns and functional relevance of identified network regulators in AD were further characterized using postmortem human brain samples and gene perturbation experiments in AD mouse models.

Results: Several key network regulators were identified as potential drivers of sex differences in AD development through unbiased systems biology approaches. A key driver gene, lipoprotein receptor related protein 10 (lrp10) as the highest-ranked key driver gene candidate in terms of regulatory strength and differential expression significance, accounting for the sex differences in AD pathogenesis and manifestation, was further validated with changes at mRNA and protein expression levels in human AD brains when compared to the aging controls. Gene perturbation experiments suggested that LRP10 differentially affected cognitive function and AD pathology in sex- and ApoE-specific manners in EFAD mouse models. Eight LRP10 binding partners were identified by the yeast two-hybrid system screening, and LRP10 over-expression reduced the association of LRP10 with one of its binding partners.

Conclusions: These findings provide insights into key mechanisms mediating sex differences in AD, which will potentially facilitate the development of sex-specific therapies for AD.
ON-DEMAND SYMPOSIUM: GENETICS OF NEURODEGENERATION 3

EXPLORING EFFECT OF KNOWN ALZHEIMER DISEASE GENETIC LOCI IN THE PERUVIAN POPULATION


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Aims: Native American populations are substantially underrepresented in Alzheimer disease (AD) genetic studies. The Peruvian (PE) population ancestry (63.6% AI, 29.7% EU, 3.8 % AF and 2.9% EA) provides a unique opportunity to assess the role of ancestry in AD. We performed whole-genome sequencing in PE case-control study to assess the effect of the known AD genes in PE population.

Methods: Whole-genome sequencing was performed in 96 AD cases and 139 unrelated cognitive healthy controls from PE population. We calculated the global ancestry (principal components) using the PC-AiR approach that is robust to known and cryptic relatedness. We tested 21 AD lead variants from the recent large non-Hispanic White (NHW) GWAS of AD (Kunkle et al. 2019). We performed association analyses with the package GENESIS, accounting for age, gender, and population substructure (first three principal components). We used Bonferroni approach for multiple test correction.

Results: Logistic regression analysis confirmed association of APOE ε4 with AD (OR=6.5, CI:3.4-12.7; pv<7.89×10⁻⁷) in PE population, but the effect size was higher than in NHW populations. Three AD loci showed nominal significance with the similar trend that was found in NHWs, which were EPHA1 (rs10808026, pv = 0.013), CLU (rs9331896, pv=0.014), and FERMT1 (rs17125924, pv=0.022) loci.

Conclusions: Our results showed that APOE ε4 significantly associated with AD in PE population. The effect of ε4 allele in Peruvians was higher than we have observed in NHW populations. Three known AD risk variants (EPHA1, CLU and FERMT1) demonstrated suggestive associations, but work is on-going to determine if these reflect true risk effects.
ON-DEMAND SYMPOSIUM: GENETICS OF NEURODEGENERATION 3

THE MONOGENIC HUB OF THE GLOBAL PARKINSON’S GENETICS PROGRAM (GP2): IN SEARCH OF NEW PD GENES

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Aims: The Global Parkinson’s Genetics Program (GP2, http://gp2.org/) is an international collaborative effort aiming to improve our understanding of the genetic architecture of Parkinson’s disease (PD). The Monogenic Hub of GP2 aims to identify novel monogenic causes of PD by performing short and long-read whole-genome sequencing (WGS) for up to 10,000 patients.

Methods:
By reaching out to PD clinicians and researchers worldwide, we have been collecting > 750 PD patients with a suspected monogenic cause. Clinical and genetic data on prescreening are obtained through the Monogenic Portal, an easy-to-use online application. All submitted cases undergo genotyping with the Neurobooster GDA (Global Diversity Array). Mutation-negative cases will be genome-sequenced based on the following selection criteria: number of (available) affected family members, consanguinity, age at onset (AAO), and a particular emphasis on currently underrepresented populations.

**Results:** We reached out to ~200 teams worldwide. With sixteen centers from 10 different countries, we started a 500-genome pilot project including ~75% familial cases and singletons with an AAO ≤ 40 years. About 20% of patients are from underrepresented populations. Data on GDA genotyping and WGS will soon be available for the first ~100 samples.

**Conclusions:** Within the Monogenic Hub of GP2, we established a pipeline to recruit unsolved PD patients with a high likelihood of carrying novel genetic causes of PD (https://monogenic.gp2.org) allowing us to prioritize these patients for comprehensive genetic analyses by WGS. This study is anticipated to contribute to the identification of novel monogenic causes of PD.
ON-DEMAND SYMPOSIUM: GENETICS OF NEURODEGENERATION 3

GENETIC PREDICTION OF IMPULSE CONTROL DISORDERS IN PARKINSON’S DISEASE

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Aims: To develop a clinico-genetic predictor of impulse control disorder (ICD) risk in Parkinson’s disease (PD).

Methods: In 5770 individuals from three PD cohorts (the 23andMe, Inc.; the University of Pennsylvania (UPenn); and the Parkinson’s Progression Markers Initiative (PPMI)), we used a discovery-replication strategy to develop a clinico-genetic predictor for ICD risk. We first performed a genomewide association study (GWAS) for ICDs anytime during PD in 5262 PD individuals from the 23andMe cohort. We then combined newly-discovered ICD risk loci with 13 ICD risk loci previously reported in the literature to develop a model predicting ICD in a Training dataset (n=339, from UPenn and PPMI cohorts). The model was tested in a non-overlapping Test dataset (n=169, from UPenn and PPMI cohorts) and used to derive a continuous measure, the ICD-Risk Score (ICD-RS), enriching for PD individuals with ICD (ICD+ PD).

Results: By GWAS, we discovered four new loci associated with ICD at p-values of 4.9e-07 to 1.3e-06. Our best logistic regression model included seven clinical and two genetic variables, achieving an area under the receiver operating curve (AUC) for ICD prediction of 0.75 in the Training and 0.72 in the Test dataset. The ICD-RS separated groups of PD individuals with ICD prevalence of nearly 40% (highest risk quartile) vs. 7% (lowest risk quartile).

Conclusions: In this multi-cohort, international study, we developed an easily-computed clinico-genetic tool, the ICD-RS, that substantially enriches for subgroups of PD at very high vs. very low risk for ICD, enabling pharmacogenetic approaches to PD medication selection.
A MULTI-STEP MODEL OF PARKINSON’S DISEASE PATHOGENESIS

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Aims: Parkinson’s disease may result from the combined effect of multiple factors. The relationship between disease incidence and age can be used to model a multistep pathogenic process, affording unique insights into disease development. We tested whether observed PD incidence is consistent with a multistep process, estimated the number of steps required and whether this varies with age, and examined drivers of sex differences in PD.

Methods: Our validated probabilistic modelling process, based on medication prescribing, generated New Zealand-wide age- and sex-adjusted PD incidence data spanning 2006-17. Models of log(incidence) versus log(age) were compared using Bayes factors, to estimate (1) if a linear relationship was present (indicative of a multistep process), (2) the relationship’s slope (one less than number of steps), (3) whether slope was lower at younger ages, and (4) whether slope or y-intercept varied with sex.

Results: Across >15,000 incident cases of PD, there was a clear linear relationship between log(age) and log(incidence). Evidence was strongest for a model with an initial slope of 5.2 [3.8,6.4], an inflexion point at age 45, and beyond this a slope of 6.8 [6.4,7.2]. There was evidence for the intercept varying by sex, but no evidence for slope being sex-dependent. Strikingly similar results were obtained from analysis of an independent UK dataset.

Conclusions: The age-specific incidence of PD is consistent with a process that develops in multiple, discrete steps – on average six before age 45 and eight after. The model supports theories emphasising the primacy of environmental factors in driving sex differences in PD incidence.
ON-DEMAND SYMPOSIUM: GENETICS OF NEURODEGENERATION

PARKINSON’S DISEASE PATIENT-SPECIFIC NEURONAL NETWORKS CARRYING THE LRRK2 G2019S MUTATION UNVEIL EARLY FUNCTIONAL ALTERATIONS THAT PREDATE NEURODEGENERATION


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Aims: To investigate early disease mechanisms occurring in Parkinson’s disease (PD) to reveal restorative targets.

Methods: Human induced pluripotent stem cell (iPSC)-derived dopaminergic neurons (DAn) obtained from healthy individuals or patients harboring LRRK2 PD-causing mutation in combination with calcium imaging, biophysical modeling, and DAn-lineage tracing using a TH-reporter iPSC line, were used to evaluate functional neuronal network activity.

Results: Here we report that human iPSC-derived dopaminergic neurons (DAn) obtained from healthy individuals or patients harboring LRRK2 PD-causing mutation can create highly complex networks with evident signs of functional maturation over time. However, LRRK2 PD patients’ neuronal networks developed abnormal hypersynchrony in the absence of neurodegeneration, in contrast to healthy or gene-edited isogenic PD networks. Moreover, we found a decrease in DAn neurite density that triggered overall functional alterations in PD neuronal networks. Thus, dysfunction of DAn physiology appears to precede the functional alterations that are then spread in the overall culture, placing DAn dysfunction as an early sign of overall alteration and neurodegeneration.

Conclusions: Taken together, our findings reveal early functional alterations that might contribute to the initiation of downstream degenerative pathways preceding DAn loss in PD, highlighting a potential window of opportunity for pre-symptomatic assessment of chronic degenerative diseases.
Aims: Cognitive decline has been shown lead to deleterious health outcomes. Diabetes is a chronic disease that exacerbates other chronic conditions and also leads to poor health. In this study, we determined whether the longitudinal evidence supports the need to monitor cognitive function in individuals diagnosed with diabetes.

Methods: We used population-based cohort study of 1999-2002 National Health and Nutrition Examination Surveys with mortality data obtained through 2015. Caucasian Americans aged 50 years or older were assessed for cognitive skills using Digit Symbol Substitution Test (DSST). Diabetes status was determined from self-reported data. Outcomes of all-cause mortality were evaluated using Cox regression and various disease levels.

Results: Percent of deaths from diabetes among the population were higher among females (12.0%) than males (9.4%). The mean follow-up was 13.1 years. For all-cause mortality, the overall unadjusted hazard ratio (HR) of diabetes to no diabetes was 2.03 (95% confidence interval [CI], 1.63-2.53, p < 0.001). Adjusted HR was elevated, 2.16 (CI 1.52-3.06, p < 0.001), among patients with low cognitive function and diabetes but closer to 1.0 (1.79 CI 1.59-2.02, p < 0.001) among patients with low cognitive function but without diabetes, after controlling for demographic risk factors.

Conclusions: Our research shows that diabetes interacts with cognitive decline. In addition, racial differences play a role in how cognitive function varies with other chronic diseases leading to increased mortality outcomes. The pathophysiology include autonomic dysfunction and neuroinflammation contributing to cognitive dysfunction. Improved identification of dementia, increased surveillance efforts, and addressing issues with health equity are needed to improve survival.
ON-DEMAND SYMPOSIUM: INSULIN SIGNALING, RISK PATHWAYS, CHARACTERIZATION OF DISEASE

THE IMPACT OF TYPE 2 DIABETES IN PARKINSON’S DISEASE

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Aims: Type 2 diabetes (T2DM) is a risk factor for developing Parkinson’s disease (PD) but its effect on disease progression is not well understood.

Methods: We investigated the effects of co-morbid T2DM on Parkinson’s disease progression. We analysed data from the Tracking Parkinson’s study, a multi-centre prospective study in the UK, which included 2006 people with recent onset PD. 167 (8.7%) patients had PD and T2DM (PD+T2DM) while 1763 (91.3%) had PD without T2DM (PD). After controlling for differences in age, sex, PD duration, ethnicity, vascular risk factors, body mass index, and Hoehn and Yahr stage, we explored how co-morbid T2DM affects PD, as proxied by comparing motor and non-motor severity scores, and undertook survival analyses using standard Cox hazards models.

Results: Patient with co-morbid T2DM were more likely to have depression (OR 1.62, CI 1.10-2.39, p=0.015), substantial gait impairment (OR 2.91, CI 1.46-5.79, p=0.002) and loss of independence (OR 2.08, CI 1.34-3.25, p=0.001) compared to patients without T2DM. Furthermore, Parkinson’s patients with T2DM had a greater longitudinal increase in both motor symptoms (p=0.012) and worsening mood symptoms (p=0.041). T2DM was also an independent predictor for the development of substantial gait impairment (HR 1.55, CI 1.07-2.23, p=0.020) and mild cognitive impairment (HR 1.7, CI 1.24-2.51, p=0.002) in patients without these symptoms at baseline.

Conclusions: As T2DM is a potentially modifiable, metabolic state, with multiple peripheral and central targets for intervention, it may represent a target for ameliorating parkinsonian symptoms and neurodegeneration, and progression to disability and dementia.
ON-DEMAND SYMPOSIUM: INSULIN SIGNALING, RISK PATHWAYS, CHARACTERIZATION OF DISEASE

A COMPARISON OF THE OPPOSING METABOLIC SIGNATURES OF ALZHEIMER’S DISEASE AND GLIOBLASTOMA

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Aims: An intriguing phenomenon has been reported in which there is an inverse relationship between the development of Alzheimer’s disease (AD) and cancer. In addition, our studies have demonstrated that CNS neoplasms are more frequent in individuals who are resistant to the development of Alzheimer’s disease. It is thought that the mechanistic pathways involved in the development of these two diseases are diametrically opposed.

Methods: Herein we examine the metabolic signatures of human AD and glioblastoma (GBM) tissue utilizing high-resolution matrix-associated laser desorption/ionization mass spectrometry imaging (MALDI-MSI).

Results: MALDI-MSI (spatial metabolomics) demonstrates several differences in the metabolism of AD and GBM tissue. Glutamate and aspartic acid are enriched in AD cortex as compared to GBM. Glutamate and aspartate are excitatory neurotransmitters that when present in excess amounts are neurotoxic and have been associated with cognitive dysfunction. In addition, phosphodimethylethanolamine levels are significantly lower in AD as compared to control and GBM. Phosphatidylethanolamine-N-methyltransferase (PNMT) enzyme activity has been reported to be reduced in regions of the brain most associated with neurodegeneration in AD, which could explain the lower levels of phosphodimethylethanolamine. In contrast, GBM displays much higher levels of 2-hydroxyglutarate as compared to the control and AD, as well as lower levels of alpha-ketoglutarate. This is to be expected given that the GBM harbors an isocitrate dehydrogenase (IDH) mutation, as do many gliomas.
Conclusions: These results highlight the dichotomy between the disease processes underlying AD and GBM. By leveraging therapeutics that target these implicated metabolic pathways, one could potentially attenuate the development of these devastating disorders.
ON-DEMAND SYMPOSIUM: INSULIN SIGNALING, RISK PATHWAYS, CHARACTERIZATION OF DISEASE

IAPP ANALOG PRAMLINTIDE REVERTS THE CONTRACTION CAUSED BY IAPP ON PERICYTES IN AN IN-VITRO MICROVASCULAR MODEL

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Aims: The blood flow in patients with Alzheimer’s disease (AD) is often reduced or stalled, a phenomenon suggested to be linked to hypercontractility of dying pericytes in presence of amyloid beta (Aβ). We have shown that brain pericytes are vulnerable to islet amyloid polypeptide (IAPP), a pancreas-derived peptide known to share properties with and seed Aβ. Our objective is therefore to use an in-vitro microvascular model to assess whether IAPP also affects pericyte contraction and whether such effect can be reversed.

Methods: Human brain pericytes were co-cultured with human brain endothelial cells on top of a basement membrane matrix until they formed vessel-like structures. The structures were then stimulated with oligomer IAPP (olIAPP) alone or in combination with pramlintide. Sphingosine-1-phosphate (S1P) and ROCK-inhibitor Y27632 were used as contraction and relaxation controls, respectively. Round-shaped pericytes were considered contracted and their proportion of total pericytes was analyzed with ImageJ software. Viability was evaluated with trypan blue staining.

Results: As expected, the proportion of round pericytes increased after S1P stimulation and decreased in response to Y27632. Also olIAPP significantly increased the proportion of round-shaped pericytes. This effect was reverted by pramlintide. Only a small subset of the round pericytes were trypan blue positive and their proportion was not significantly altered after olIAPP stimulation.

Conclusions: Our results indicate that IAPP induce hypercontraction in pericytes, which may contribute to the stalled blood seen in AD patients. Pramlintide could be a candidate drug to prevent IAPP-induced pericyte hypercontraction.
ON-DEMAND SYMPOSIUM: INSULIN SIGNALING, RISK PATHWAYS, CHARACTERIZATION OF DISEASE

UNCOVERING A ROLE FOR GBA1 IN HOST RESPONSE AS INFORMED BY PARKINSON DISEASE RISK

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Aims: Typical Parkinson disease (PD) is a multi-factorial disease, caused by a contribution of genetic and environmental factors. PD pathology has been hypothesized by Braak and colleagues to start in the periphery in the gut and olfactory system by exposure to pathogens, and subsequently progress towards the midbrain and cortex along olfactory and vagus nerve tracts in a staged manner. In this vein, we have sought to identify roles for PD-associated risk genes in response to microbial infection. The goal herein was to identify how Gba1, the commonest PD risk gene, is involved in host response to infection.

Methods: C57Bl/6J mice carrying p.D409V knock-in mutations were challenged with selected pathogens, based on their tropism and their elicited immune responses. Microbial insults targeting the spleen (Salmonella typhimurium), lung (mouse adapted Influenza A H1N1) and brain (vesicular stomatitis virus) were chosen. Survival, microbial load, sickness outcomes and GCase activity were measured to assess pathogen- and tissue-specific effects of Gba1 in host responses.

Results: Mutations in Gba1 appear to alter infectious outcomes in response to neurotropic VSV infection in a sex- and genotype-dependent manner. Heterozygous mice survive at increased rates compared to their wild type littermates, and a homozygous survival benefit shows a sex-bias in males. In response to VSV infection, GCase activity is selectively altered in an organ- and sex-dependent manner. Lipid analysis is ongoing.

Conclusions: This study highlights a role for Gba1 in protection against pathogens and provides potential insight into disease mechanisms, based on the testing of Braak’s hypothesis.
ON-DEMAND SYMPOSIUM: INSULIN SIGNALING, RISK PATHWAYS, CHARACTERIZATION OF DISEASE

CAN OLFACTORY DEFICITS, COGNITIVE MEASURES, AND RESTING STATE FUNCTIONAL CONNECTIVITY SERVE AS PRECLINICAL MARKERS IN PATIENTS AT RISK FOR DEVELOPING PARKINSON’S DISEASE?

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Aims: Hyposmia is one of the most common non-motor symptoms of Parkinson’s disease (PD), often appearing before PD is diagnosed. However, hyposmia is not specific to PD, and additional markers are needed to identify PD in its earliest stages. We examined whether specific cognitive deficits and/or abnormalities in resting-state functional connectivity (rsFC) within the default mode network (DMN) may serve as additional markers of PD for at-risk individuals.

Methods: Patients with PD (n=26), healthy controls (n=26), and an at-risk group (AR) including hyposmic first-degree relatives of PD patients and unrelated hyposmic individuals (n=30) were compared on rsFC of the DMN and neuropsychological tests.

Results: Relative to controls, the AR group demonstrated lower scores on neuropsychological tests of verbal working memory. PD patients also exhibited processing speed deficits. On MRI, the AR groups showed increased rsFC between the anterior medial prefrontal cortex and the right middle temporal gyrus of the DMN compared to both the control and PD groups.

Conclusions: Trials aimed at the prevention of PD require identification of those most at risk of its development. Individuals who are at higher risk of developing PD demonstrated poorer verbal working memory and increased DMN rsFC relative to healthy controls while PD patients only differed from controls on cognitive testing. Alterations in DMN rsFC for persons at risk of PD may indicate early compensatory processes in the face of ongoing neurodegeneration and progression towards PD. Longitudinal studies should examine rsFC, working memory and processing speed as potential indicators of increased risk for PD development.
DUAL-TASK REHABILITATIVE TRAINING IN CORONARY ARTERY BYPASS GRAFT SURGERY PATIENTS

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Aims: The dual-task (DT) training is a relatively new method that involves a cognitive task combined with postural and walking control. Aim of the study was to assess the effects of a dual task rehabilitative training on neurophysiological parameters in patients after on-pump coronary artery bypass graft (CABG) surgery.

Methods: Forty eight CABG patients were randomized using envelopes to DT training group (n = 23) and non-training group (n = 25). An extended neurophysiological assessment (psychometric tests and electroencephalogram study) and stabilography 3-5 days before and 8-11 days after coronary artery bypass grafting were conducted. DT training was carried out daily, starting 3–4 days after the procedure and until the discharge order. The training itself consisted of a cognitive task combined with postural control, and lasted 15-20 minutes.

Results: The DT training patients had postoperative cognitive dysfunction (POCD) in 39% cases, while the non-training group - in 64%. The relative risk of POCD in the non-training group was 2.77 (95% CI: 0.86–8.91, Z = 1.704, p = 0.08). Only DT training patients exhibited better cognitive state compared to the preoperative state (Z = 2.58; p = 0.01). Theta power values increased in the non-training group in comparison to the preoperative values, while the DT training group did not have a statistically significant difference in theta power.

Conclusions: Positive effects of dual task rehabilitation on the neurophysiological parameters of CABG patients included lower frequency of POCD, improved cognitive state and less pronounced cortical dysfunction. DT training had proved itself a suitable training method for these patients.
Aims: Down syndrome (DS) is characterized by neurological deficit, which includes cognitive dysfunctions that are associated with molecular alterations also frequent in Alzheimer’s disease (AD), such as the accumulation of amyloid β protein, the onset of insulin resistance in the brain and the over-expression of microRNAs (miRNAs). In particular, miR-802, encoded on chromosome 21, seems to play a key role in the insulin signaling pathway in metabolic diseases. Based on these evidence, the aim of the work was to analyze the expression of miR-802 in DS and its role in the alterations of the insulin signal in the brain.

Methods: We evaluated the gene expression of miR-802 on prefrontal cortex taken from subjects with DS and related controls and from a mouse model of DS (Ts65Dn, at 3-9 months). Then, through a bioinformatics analysis we focused on target genes of miR-802 that encode proteins of the insulin signaling pathway (PTEN and GSK-3β).

Results: The results obtained show a high expression of miR-802 in subjects with DS compared to controls and in the brains of Ts65Dn mice at 9 months of age. The over-expression of miR-802 is associated with a reduction in the mRNA and protein levels of GSK-3β, both in subjects with DS and in Ts65Dn mice. Furthermore, in vitro experiments support the role of miR-802 in the regulation of GSK-3β expression.

Conclusions: These results suggest that increased levels of miR-802 expression are evident in DS brain and promote a reduction in GSK-3β expression, known for its role in insulin signaling and in the regulation of synaptic plasticity.
ON-DEMAND SYMPOSIUM: INSULIN SIGNALING, RISK PATHWAYS, CHARACTERIZATION OF DISEASE

TRAJECTORIES OF ACTIVITIES OF DAILY LIVING IN PERSONS WITH DEMENTIA

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Aims: Objectives: In a longitudinal cohort of dementia patients, we constructed trajectories of basic and instrumental activities of daily living (BADL and IADL) using demographics and global cognition as determinants of decline, and explored differences in white matter hyperintensity volume (WMH), APOE-ε4 genotype and neuropsychiatric symptoms amongst different trajectories.

Methods: 502 patients were included with brain MRI, APOE genotype, Mini-Mental State Examination (MMSE), Neuropsychiatric Inventory (NPI), and Disability Assessment in Dementia. Using latent-class trajectory modelling, we calculated trajectories of BADL and IADL using ADL assessments over five annual visits adjusting for age, sex, education, and MMSE at baseline. We then compared WMH volume, APOE-ε4, and NPI score at baseline between the identified trajectory groups.

Results: For BADL, we identified four trajectories: maintaining functionality throughout (maintainers1), maintaining functionality until fourth follow-up (maintainers2), starting high on functionality and declining gradually (gradual decliners), and starting moderate on functionality but declining quickly (fast decliners). Compared to maintainers1, gradual decliners (β: 6.62, 95% CI:2.73-10.5) and fast decliners (β: 3.70, 95% CI:0.002-7.40) had significantly higher NPI scores at baseline. For IADL, we identified four trajectories: maintenance of functionality (maintainers), starting high on functionality but declining (high decliners), starting moderate on functionality but declining (intermediate decliners) and starting low on functionality and declining (low decliners). NPI score at baseline was significantly higher in intermediate- (β: 4.61, 95% CI:2.96-6.23) and low decliners (β: 7.89, 95% CI:5.64-10.1) compared to maintainers. APOE-ε4 and WMH did not contribute to both analyses.
Conclusions: In a heterogeneous group of dementia patients, neuropsychiatric symptoms were a significant determinant for declining function on both BADL and IADL.
ON-DEMAND SYMPOSIUM: CHARACTERIZATION OF DISEASE, PATIENT CARE AND SUPPORT

UNRAVEL THE HETEROGENEITY WITHIN ALZHEIMER’S DISEASE

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Aims: To understand the heterogeneity in AD and define biologically meaningful subtypes has recently gained attention as a driver of precision medicine and for future clinical trials. Here, I want to give an update on where we stand today, present new findings and give some future directions.

Methods: The field is still missing longitudinal studies to model disease subtypes over time. Further, research has been dominated by subtypes from structural MRI, which may be less sensitive to the earliest brain changes. Other imaging modalities need to be further tested for the subtyping of the future. Cross-modal comparisons and multimodal subtyping studies are still very limited. To fully understand the complexity and heterogeneity within AD, we need to model subtypes leveraging on multimodal imaging and machine learning considering the complex relationship between protective/risk factors and concomitant non-AD pathologies.

Results: I will give a short update on where we stand today and present new data which address the limitations that are currently hampering the field (longitudinal studies and cross-modal comparisons etc.). As an example, our preliminary data show that localization and longitudinal atrophy change vary differentially across different patterns of tau-PET, indicating that the conventional idea of atrophy being a downstream event of tau is not true for all subtypes.

Conclusions: By disentangling the heterogeneity in AD, will in the future result in more personalized medicine, which is of great importance with emerging disease modifying treatments and for the recruitment of participants for successful drug trials.
ON-DEMAND SYMPOSIUM: CHARACTERIZATION OF DISEASE, PATIENT CARE AND SUPPORT

QUADRATO MOTOR TRAINING AS A POSSIBLE TOOL FOR SENSORIMOTOR COGNITIVE TRAINING IN NEURODEGENERATIVE STATES

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Aims: Finding efficient ways of activating the body’s resources through cognitive and physical activity in neurodegenerative states is important. Recent studies reported that both cognitive and physical exercise can, at least partly, reduces the risk of cognitive decline. Combining a training which includes both a strong cognitive and sensorimotor activation can have a synergetic effect. Consequently, we chose to examine the Quadrato Motor Training on mild-cognitive impaired participants and Alzheimer patients (AD). Given that 1) many neurodegenerative patients, such as AD, suffer from reduced alpha activity (10 Hz) and that 2) QMT was found to enhance alpha activity and cognitive functions related to AD, 3) we hypothesized that QMT could improve healthy EEG markers in neurodegenerative states.

Methods: We used a pre post resting state EEG design to examine the electrophysiological state of the participants before and following a month of daily training. A simple walking training provided a control condition using the same auditory stimuli and motor performance (steps in one room of own home) as QMT but with minimal cognitive demands.

Results: The finding supporting the ameliorating effects of QMT on the disease progression, by enhancing healthy neuronal synchronization and improving coordination.

Conclusions: In conclusion, it is possible intervene to slow down the cognitive decline by promoting physical and cognitive trainings, both in case of AD or healthy ageing. QMT is a training that have been linked to improvements in cognitive, affective, physiological and functional domains. QMT could be a valuable tool for slowing down cognitive decline of neurodegenerative disorders.
ON-Demand Symposium: Characterization of Disease, Patient Care and Support

Online self-management and health promotion in early-stage dementia with e-learning for carers – SHAPE. A multicentre, randomized, controlled trial.

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Aims: The SHAPE trial will include 336 people with mild to moderate dementia to evaluate the effectiveness of a group-based online educational programme. The main objective is to determine whether the SHAPE intervention will significantly improve self-efficacy in people with mild to moderate dementia. Secondary objectives are the impact on mood, function, wellbeing and quality of life, health behaviours, cognition, neuropsychiatric symptoms, carer stress, knowledge of dementia, and cost effectiveness in people with dementia and their care partner.

Methods: This is a multi-site, controlled, single block-randomised, single-blinded trial with parallel arms. The intervention compared with treatment as usual (TAU). Outcome measures will be reported at baseline, after the intervention (follow-up 1) and 6-months after follow-up 1 (follow up 2). The primary outcome (self-efficacy) and secondary outcomes will be analysed using multilevel modelling to analysis of covariance, with 95% confidence interval and associated p-value. We will calculate incremental cost-effectiveness ratios of the intervention as difference in costs between SHAPE and TAU over the difference in outcomes between groups, for each outcome in turn.

Results: We hypothesise that the intervention will significantly improve self-efficacy, wellbeing and quality of life, and other key outcomes (e.g. depression, anxiety, produce health promotion behaviour and quality of life) for people with dementia; reduce carer stress, increase knowledge of dementia; provide a cost-effective approach to improve wellbeing and quality of life, compared to treatment as usual.

Conclusions: We will assess the effectiveness of a group-based online educational programme combining approaches of self-management and health promotion for persons with early stage dementia.
WORRY AND RUMINATION’S ASSOCIATION WITH COGNITIVE HEALTH AND PHYSICAL HEALTH IN OLDER ADULTS AT RISK OF DEMENTIA

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Aims: Worry and rumination have been proposed as mechanisms that influence cognitive (e.g. risk for Alzheimer’s disease) and physical health (e.g. risk for cardiovascular disease) in older adults. We aimed to determine whether worry and rumination are related to subjective perception and/or objective measures of health in older adults at risk of dementia.

Methods: Baseline data from 141 participants from the SCD-Well trial were used (91 female; M_age=72.7 years). Rumination and worry were measured using the Ruminative Response Scale brooding subscale and the Penn State Worry Questionnaire, respectively. Subjective physical health was assessed using a physical quality of life measure (WHOQoL-Bref subscale), and objective physical health via modified versions of the Framingham Risk Score and Charlson Comorbidity Index. Subjective and objective cognition were assessed using the Cognitive Difficulties Scale and a modified Preclinical Alzheimer’s Cognitive Composite, respectively. Linear regressions, adjusted for education, age, and sex, were conducted.

Results: Worry and rumination were negatively associated with subjective physical (worry: β=-0.10, p=0.005; rumination: β=-0.49, p=0.002) and cognitive (worry: β=-0.01, p=0.047; rumination: β=-0.09, p=0.007) health. No associations were observed between worry or rumination and objective physical or cognitive health.

Conclusions: Worry and rumination may be common mechanisms associated with subjective but not objective physical and cognitive health. Alternatively, cognitively unimpaired and physically healthy older adults may become aware of subtle changes which are not yet captured by objective measures. Interventions which reduce worry and rumination may promote subjective physical and cognitive health. More research is needed to determine causality of the relationships in this study.
ON-DEMAND SYMPOSIUM: CHARACTERIZATION OF DISEASE, PATIENT CARE AND SUPPORT

EHR COHORT DEVELOPMENT USING NATURAL LANGUAGE PROCESSING FOR IDENTIFYING SYMPTOMS OF ALZHEIMER’S DISEASE

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Aims: Alzheimer’s Disease (AD) is the most common reason for neurodegenerative dementia. Patients with AD may suffer from memory loss, neuropsychiatric and behavioral diseases such as depression, anxiety and apathy, poor judgment, as well as speaking, walking and swallowing difficulties, some of which are not encoded in the structured data of electronic health records (EHRs), but are described in the EHR notes. In this study, we built natural language processing (NLP) models that automatically identify the aforementioned symptoms of mild, moderate, and severe AD.

Methods: We first expert-annotated 3,000 sentences containing a list of keywords commonly used for AD symptoms, e.g., “memory loss” and “apathy.” Those sentences were selected from EHR notes of patients diagnosed with AD in the US Department of Veterans Health Administration (VHA). We annotated each sentence to contain either AD symptom or not. Using those 3,000 annotated sentences as training and testing data, we developed NLP models to automatically classify whether a sentence contains an AD symptom.

Results: Our annotation agreement is 0.81 Cohen’s kappa. Our best NLP model is built on the deep learning Bidirectional Encoder Representations from Transformers model pre-trained on the VHA EHR notes of 8 million patients, achieving an F1-score of 0.91 based on 10-fold cross-validation. Using this NLP model, we built a large AD symptom (ADS) cohort of over 2 million patients at VHA where AD symptoms identified by NLP are linked to their structured EHR data.

Conclusions: The next step is to determine how these detected symptoms are related to an AD diagnosis.
Aims: Risk factors have been identified for progression to cognitive impairment and neurocognitive disorders. These include loneliness, feelings of belonging, engagement in cognitive stimulation, Mediterranean-like diets, avoidance of diabetes, exercise, antioxidants, having larger social circles, and engaging in stress reduction activities. Protocols exist for preventing and reducing cognitive impairment.

Methods: We designed a system's dynamics computer simulation model to predict the onset of cognitive impairment given the course of a person's life history up to the moment in time of measurement. The simulation model incorporated the role of exercise, genetic load, age, quality of diet, presence of diabetes and level of hemoglobin A1C, ongoing levels of cognitive stimulation, presence or absence of micronutrients, presence or absence of other co-morbidities, overall general health index, levels of smoking and other substance use, and family history.

Results: The model was built with data from 10 individuals. Then we entered data from another 10 people to assess accuracy for ten new individuals for whom it had not been developed. Success was a prediction of onset within 10% of the actual date. We had 7 successes. We then modeled an additional 10 people, asking them what they would be willing to change to alter their predictions. We then re-ran the model using the changed variables to show what difference altering these factors could make.

Conclusions: We conclude by discussing the difficulties inherent in people changing their modifiable risk factors, such as diet, exercise, learning new information, becoming more social, learning stress reduction techniques, sharing some tools of persuasion.
ON-Demand Symposium: Characterization of Disease, Patient Care and Support

Sniffin’ Stick Test Upgraded Protocol for Russian Population Yields Efficient Differentiation in Diagnostics of Parkinson’s Disease and Essential Tremor


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Aims: Improvement of diagnosis accuracy of Sniffin’ Stick Test (SST) through adaptation of original protocol to Russian language population of conditionally health people and the patients with Parkinson’s disease (PD) and essential tremor (ET).

Methods: We used the standard Sniffin’ sticks test (BurghartMesstechnik, Germany) consisting of three subtests. Statistical analysis and treatment of the data were provided by SPSS Statistics 23 workbench. The data collected over the patients were tested with Shapiro-Wilk test for normality; the groups of the patients were compared with Mann-Whitney and Student tests. Visualization and non-linear clustering were provided by freely distributed VidaExpert software.

Results: The results of the first subtest obtained due to standard protocol failed to distinguish the groups of patients. The protocol was modified by randomizing the order of smelling triplets exposition; the modification greatly improved clustering. Also, the standard protocol of the third subtest revealed inferior recognition of the smell agent names due to a low test localization level. We measured the unattended knowledge of smelling agent names to modify the third subtest protocol to improve the situation. Namely, we chose the highest-ranked names mentioned by respondents.

Conclusions: Randomization of the smelling triplets exposure in the first subtest significantly improved the differential diagnostics of PD patients from those with ET. Sounding disadvantages of the third subtest had been revealed, and the ways to improve the protocol were proposed. Also, the new A-test was implemented based on the particular procedure of smell recognition from the set of four ones.
Aims: The electronic medical record (EMR) is an effective technological tool that can be utilized in the evaluation of patient data and epidemiological trends. Central New York (CNY) is a diverse ethnic area. The largest proportion of the population includes Caucasian, African American (AA), American Indian (AI), Asian, and Native Hawaiian/Other Pacific Island (NH/OPI) residents. The goal of this study is to identify demographical disparities in the evaluation and/or treatment of neurodegenerative disorders among the CNY population.

Methods: A 2012-2020 patient cohort with neurodegenerative disorders was studied. A patient cohort of Alzheimer’s disease (AD), vascular dementia (VaD), mild cognitive impairment (MCI), dementia with Lewy bodies (DLB), frontotemporal dementia (FTD), Parkinson’s disease (PD), parkinsonism, multiple system atrophy (MSA), corticobasal degeneration (CBD), and progressive supranuclear palsy (PSP) diagnoses was constructed using the EPIC EMR platform. Data was analyzed and presented graphically using Microsoft Excel.

Results: The cohort of 13,278 patients with neurodegenerative disorders included 4,662 (35%) AD, 2,096 (16%) VaD, 2,767 (21%) MCI, 297 (2%) DLB, 196 (1%) FTD, 2859 (22%) PD, 274 (2%) Parkinsonism, 45 MSA (0.4 %), 13 (0.1%) CBD, and 69 (0.5%) PSP. Subgroup analysis was conducted for each ethnicity.

Conclusions: Data elicited from EMRs wholly reflect populations, thus providing opportunities to discern healthcare discrepancies and improve outcomes. In addition, our study indicates that the incidence and correlation are in agreement with reported literature. Therefore, our dataset can be part of multicenter trials due to similar presentation and cohort.
ON-DEMAND SYMPOSIUM: CHARACTERIZATION OF DISEASE, PATIENT CARE AND SUPPORT

VOICE FEATURES OF COGNITIVE LOAD DURING DUAL TASKING

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Aims: Older adults show reductions in gait speed when performing a concurrent cognitive task, an effect termed dual-task interference, but less is known about changes to the concurrent task. Here we describe accuracy, timing, and acoustic features of speech during performance of a cognitive task under dual task conditions.

Methods: 11 participants aged 26-65 years took part in two sessions where they completed serial subtraction by three and seven (low vs high load) while sitting or walking (single or dual task). Voice data was captured using the NeuroVocalix system and transcribed. Timing, accuracy, and low-level acoustic features were extracted for analysis from each attempt. Gait data were captured but are not reported here. Machine learning gradient boosting classification models with leave one group out cross validation were applied to acoustic data.

Results: Overall subtraction accuracy was worse under conditions of high cognitive load (23% vs 13% error) but unaffected by dual tasking. Similarly, timing was primarily affected by cognitive load. Using acoustic features, we predicted cognitive load condition with an average accuracy of 78% and an average area under the curve (AUC) of 0.86. For models of dual task condition (walking vs sitting) a mean accuracy of 79% and an AUC of 0.86 were obtained.

Conclusions: Although accuracy was unaffected by a concurrent walking in this sample, acoustic features are able to distinguish performance under single vs dual task conditions and low vs high cognitive load, suggesting the utility of voice capture while dual-tasking in enhancing the sensitivity.
Aims: To demonstrate whether dual-task training with Action Observation Training (AOT) and Motor Imagery (MI) ameliorates cognitive performance and resting-state (RS) brain functional connectivity (FC) in Parkinson’s disease (PD) patients with postural instability and gait disorders (PIGD).

Methods: 20 PD-PIGD patients were randomized into 2 groups: i) DUAL-TASK+AOT-MI group performed a 6-week training consisting of AOT-MI combined with practicing observed-imagined gait and balance exercises; ii) DUAL-TASK group performed the same exercises combined with landscape-videos observation. All patients underwent a computerized cognitive assessment and RS-fMRI scans at baseline and after training. Cognitive and RS-FC changes (and their relationships) over time within and between groups were assessed.

Results: After training, all PD-PIGD improved in terms of accuracy and reaction times in test assessing executive-attentive (mainly dual-task) skills. The within-group analyses showed that: the DUAL-TASK+AOT-MI group had increased RS-FC within the Anterior Salience Network (aSAL), the right Executive Control Network and the Precuneus, and reduced RS-FC within the anterior Default Mode Network (aDMN); while the DUAL-TASK group showed increased RS-FC within the Visuospatial Network. GroupxTime interactions showed that, compared to the DUAL-TASK group, the DUAL-TASK+AOT-MI group showed increased RS-FC within the aSAL, which correlated to reduced response latency, and reduced RS-FC within the aDMN, which correlated to better accuracy in this group.

Conclusions: In PD-PIGD patients, both trainings promote cognitive improvement and brain functional reorganization. The DUAL-TASK+AOT-MI training is further useful for obtaining specific functional reorganization of extra-motor brain networks involved in motor control and executive-attentive abilities with specific effects on dual-task mobility and balance.
Aims: Cognitive impairments seen in Parkinson's disease (PD) are pervasive and can be seen in many domains, including decision-making. When uncertain, effective decision-making results from combining external sensory information with internal information (memories of past experiences known as priors). Remarkably little is known about the underlying neurocircuit of memory-based decision-making impairment in PD and its relationship with dopamine.

Methods: PD patients on and off dopaminergic medication and healthy controls (HCs) performed a decision-making task in which reported the orientation of a Glass pattern made from green or red dot pairs and received feedback. The coherence of the dot pairs was randomly varied, changing the difficulty of the orientation discrimination. To assess the effect of memory on decision-making, the likelihood of one orientation for one of the two colored patterns was varied.

Results: HCs and PD participants were more accurate when the coherence of the dot pairs was high, and they guessed when coherence was low. HCs but not PD participants (off dopaminergic medication) could use priors to make correct decisions when coherence was low. However, PD participants' ability to use priors in response to dopamine was heterogenous, alluding to individual differences.

Conclusions: Decision-making performance degrades in PD when priors must be combined with sensory information to guide decisions. We suggest this process relies on the healthy connections between structures involved in memory (medial temporal lobe) and decision-making (basal ganglia and frontal cortex). Furthermore, the effect of dopamine on memory-based decision-making varies, which can be explained by different subgroups of PD (akinetic-rigid versus tremor-dominant).
Aims: Amyloid precursor protein (APP) plays a pivotal role in Alzheimer’s disease (AD), which is associated with synaptic failure. Therefore, our goal is to unravel APP physiological function at the glutamatergic synapse to better understand AD pathology, in particular its role in nascent synapses and synaptic maturation.

Methods: Since synaptic maturation varies throughout time, we have studied APP function in wild-type mice hippocampus during development, adulthood and aging. Moreover, we have correlated APP function with synaptic maturation, by categorizing synapses based on their NMDA receptor (NMDAR) subunit composition. While immature synapses contain predominantly GluN2B-NMDARs, mature synapses exhibit mostly GluN2A-NMDARs.

Results: We have observed a predominance of ‘immature’ synapses and a peak in APP levels in infant mice, consistent with APP recruitment to nascent synapses. On the other hand, aging is associated with an increase in APP processing, that could compromise its function at the synapse. Consistently, the synaptic profile becomes more ‘immature’ upon aging, suggesting a deficit in synaptic maturation. Therefore, our data suggests that APP function correlates with synaptic maturation. We propose a model in which APP stabilizes GluN2B-NMDARs at the synapse and their consequent activation promotes synaptic maturation. Interestingly, we found an interaction between APP and GluN2B, which proved to be crucial to maintain GluN2B-NMDAR synaptic contribution.

Conclusions: We have described a novel role for APP as a stabilizing agent of GluN2B-NMDARs at the synapse. We propose that this process is essential during development/adulthood to induce synaptic maturation but becomes dysregulated upon aging, contributing to synaptic impairment.
ON-DEMAND SYMPOSIUM: APP, APLP, AMYLOIDS, BACE-1, PRESENILIN: CELL, MOLECULAR, METABOLOMICS, TRANSCRIPTOMICS AND SYSTEMS BIOLOGY

METABOLOMICS PROFILING REVEALS DISTINCT SIGNATURES IN THE SERUM AND BRAIN METABOLOMES IN MOUSE MODELS OF ALZHEIMER'S DISEASE

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Aims: Progress in development of efficacious therapies for Alzheimer’s disease (AD) is halted due to limited understanding of underlying pathological mechanisms. Increasing evidence suggests that metabolic impairments prior to symptom development can contribute to disease mechanisms and subsequent dementia. Signals in conserved metabolomic pathways could provide a method to translate experimental biomarkers in preclinical mouse models to humans.

Methods: We investigated the sex-stratified associations of serum and brain metabolites from APOE4.Trem2R47H and the 5xFAD mouse models at six months of age. Metabolites were measured with targeted metabolomics.

Results: We identified a sex-specific increase of glycerophospholipids levels in male mice, while levels of sphingolipids were more abundant in females. Further, we identified that serum levels of glycerophospholipids were reduced in APOE4.Trem2R47H mice compared to C57BL/6J controls, while levels of these metabolites in the same animals were greater in both male and female brains of APOE4.Trem2R47H and 5xFAD mice. Several of these findings were consistent with recent results from the ADNI and ROSMAP cohorts, which suggested a similar decrease in the same metabolites in serum of APOE4 carriers and indicates a serum-based effect of APOE genotype. We simultaneously observed an increase in the same metabolites in brain, consistent across humans and mice.

Conclusions: Metabolomic signatures were notably different between brain and serum in mouse models. The 5xFAD mice exhibited stronger effects in brain, whereas the APOE4.Trem2R47H mouse showed more pronounced effects in serum. These findings are consistent with high levels of amyloid pathology in 5xFAD mouse brains and the modifications of serum biomarkers in APOE4.Trem2R47H mice.
ON-DEMAND SYMPOSIUM: APP, APLP, AMYLOIDS, BACE-1, PRESENILIN: CELL, MOLECULAR, METABOLOMICS, TRANSCRIPTOMICS AND SYSTEMS BIOLOGY

CALPAIN-DEPENDENT CLEAVAGE OF FERMT2 INHIBITS ITS IMPACT ON APP METABOLISM

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Aims: Genome-wide screening suggested a large proportion of the GWAS-defined AD genetic risk factors that also modulate APP metabolism are involved in the focal adhesion signaling. Among these, FERMT2 is particularly interesting because we found that decreased FERMT2 (kindlin-2) expression altered both axonal growth and long-term potentiation in an APP-dependent manner. This study aims to identify molecular mechanisms by which FERMT2 is involved in synaptic signaling, focusing on calcium-dependent processes.

Methods: We induced excitotoxicity in primary hippocampal neurons and in mouse brains via calcium treatment to investigate potential cleavage of FERMT2 in the context of neurotoxicity. We used co-immunoprecipitation and immunoblotting to analyze the impact of calcium on FERMT2 cleavage and to identify binding partners of FERMT2 cleavage products. We used HEK-APP cell lines to study the consequences of these molecular interactions on APP metabolism.

Results: We observed calpain-dependent cleavage of FERMT2 after the induction of excitotoxicity. Although this cleavage did not affect directly the molecular interaction between FERMT2 and APP, we observed that it abolished FERMT2’s capacity to regulate APP metabolism. Additionally, we demonstrated that calpain-dependent cleavage inhibits FERMT2’s physiological functions by limiting its ability to recruit c-SRC, which has been recently identified as a genetic risk factor of AD and a strong modulator of APP metabolism.

Conclusions: Altogether, our data demonstrate that the cleavage of FERMT2 by calpain has a detrimental effect on FERMT2 function. The functional interaction between FERMT2 and c-SRC, two key regulators of focal adhesion pathway, suggests an important role of this pathway in the control of APP metabolism.
CIRCADIAN RHYTHMICITY OF BETA-AMYLloid SCAVENGERS IS DISRUPTED IN THE CHOROID PLEXUS OF AN ALZHEIMER'S DISEASE MOUSE MODEL

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Aims: The choroid plexus (CP), which constitutes the blood-cerebrospinal fluid barrier (BCSFB) was recently described as an important component of the circadian clock system. The CP is the principal source of cerebrospinal fluid (CSF) and is also responsible for the synthesis and secretion of various proteins involved in beta-amyloid (Abeta) transport/degradation, contributing to Abeta homeostasis. Inadequate Abeta metabolic clearance and transport across the BCSFB has been associated with circadian disfunctions in Alzheimer's disease (AD) patients. We wanted to investigate whether AD pathology can alter Abeta scavengers' circadian expression, if Abeta scavengers' circadian expression was affected by Abeta treatment in a human CP papilloma (HIBCPP) and finally analyze the involvement of circadian rhythm in Abeta uptake.

Methods: We collected CP at different time points from an AD mouse model (APP/PS1) (female and male animals, aged 6- and 12-months-old) and analyzed the mRNA expression by Real-time RT-PCR. HIBCPP cell line was treated with Abeta and Abeta scavengers mRNA expression evaluated by Real-time RT-PCR. HIBCPP cell line was treated with Abeta-488 and uptake evaluated at different time points using flow cytometry.

Results: Only angiotensin converting enzyme (Ace) expression in 6-month-old female Wild Type mice and tranthyretin (Ttr) expression in 12-month-old female Wild Type mice presented significant rhythmicity. Circadian rhythmicity of clusterin (Clu) expression was lost in Abeta treated cells when compared to the non-treated cells. Abeta uptake displayed a circadian rhythmicity.

Conclusions: Our results suggest that AD might affect Abeta scavengers' rhythmicity and that Abeta clearance is a rhythmic process possibly regulated by Abeta scavengers' rhythmic expression.
INHIBITION OF AMYLOIDOGENESIS BY NEURONAL AQUAPORIN 1 THROUGH MODULATION OF THE INTERACTION BETWEEN AMYLOID PRECURSOR PROTEIN AND BETA-SECRETASE

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Aims: Alzheimer’s disease (AD) is characterized by amyloid beta accumulation. The AQP family of membrane water channel proteins include Aquaporin 1 (AQP1). In the early stages of AD, an increase in the level of cortical AQP1 has been found, but its role remains to be elucidated.

Methods: Brain samples from AD patients and transgenic mice (3xTg-AD and 5xFAD) were used to evaluate the expression patterns and distribution of AQP1.

Results: Cortical neurons from AD patients, and 3xTg and 5xFAD mice neurons from the affected regions showed accumulation of AQP1. In AD mice models, there was a positive correlation between aging and AQP1 expression levels. The response to stress through Trophic factor withdrawal (TFW) was evaluated in primary neuron cultures and revealed that stress induces the translocation of AQP1 to beta and gamma secretase endocytic compartments. Human neuroblastoma cells overexpressing mutated amyloid precursor protein (APP) with the Swedish mutations were used to express AQP1, and a reduction of beta secretase 1 (BACE1) mediated cleavage of APP and reduced amyloid beta production were found. By contrast, enhanced amyloid beta production and increased BACE1 activities were induced by AQP1 knockdown. Decreased association of APP and BACE1 by AQP1 was demonstrated by immunoprecipitation. A negative correlation between the expression level of AQP1 and the accumulation of amyloid beta was found through human database analysis.

Conclusions: This study suggests that stress induces upregulation of AQP1 in neurons resulting in a reduction of amyloid beta production through the modulation of BACE1-APP interactions.
ON-DEMAND SYMPOSIUM: APP, APLP, AMYLOIDS, BACE-1, PRESENILIN: CELL, MOLECULAR, METABOLOMICS, TRANSCRIPTOMICS AND SYSTEMS BIOLOGY

ELUCIDATING THE MOLECULAR BASIS OF ALTERED PRESENILIN 2 EXPRESSION ON ALZHEIMER’S DISEASE PATHOLOGY

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Aims: Objectives: γ-Secretase is an intramembrane protease which plays a pivotal role in the onset and progression of Alzheimer’s disease (AD). Presenilin provides the catalytic activity of which two homologues exist, Presenilin-1 and Presenilin-2 (PSEN1/2). PSEN2/γ-secretase complexes are restricted in their localization to the late endosomes and lysosomes, as opposed to the broader distribution of PSEN1/γ-secretase, making it the main generator of intracellular abeta (Aβ). Although the focus has been on the extracellular pool, this toxic intracellular pool has been shown to precede plaque and tangle formation and correlates well with synaptic dysfunction highlighting its importance. This project aims to unravel the effects of altered PSEN2 expression and this intracellular pool on AD pathogenesis.

Methods: We generated novel mouse models by crossing the well characterized APP NL-G-F model with a PSEN2 knock out (KO) mouse and an in-house generated PSEN2 knock in (KI) model carrying the familial AD-linked N141I mutation (FAD-PSEN2).

Results: Curiously, for both genotypes, we found an identical accelerated plaque pathology. These alterations in pathology equally translated to cognitive deficits. In contrast, gliosis was markedly different with increased and earlier microglia recruitment in the case of PSEN2KO whereas APP KlxFAD-PSEN2 displayed a delayed recruitment.

Conclusions: Conclusion: These differential effects on distinct pathological features suggest both a protective role for PSEN2 as well as specific, yet unexplored, roles in neurons as well as glial cells. We are currently examining primary neurons and microglia from the different genotypes to explore underlying molecular mechanisms.
Aims: The presenilin protein homologues, presenilin-1 and presenilin-2, are most well known as the catalytic component of gamma-secretase, which generates amyloid-beta (Aβ) peptides. In the context of Alzheimer’s disease, presenilin-1 has garnered greater focus, at the detriment of understanding the structural and functional differences between the two presenilin homologues. The objective of this project is to identify the specific contributions of presenilin-1 and presenilin-2 to Aβ metabolism, including its roles in both generation and removal of Aβ.

Methods: This study integrates computational modelling and in vitro experiments, using novel human-derived presenilin knockout cell lines generated from M17 and HMC3 cells, to evaluate expression, substrate processing activity, binding affinity and stability, and cellular processes to generate and remove Aβ.

Results: We developed a technique to enable quantitative assessment of presenilin-1 and presenilin-2 protein expression. We show that when expression is considered, presenilin-2 gamma-secretase processes 80% more amyloid precursor protein than presenilin-1 gamma-secretase, while Notch processing by both presenilin gamma-secretase complexes is equal. Computational analysis suggests differences in substrate binding between presenilin-1 and presenilin-2 gamma-secretase complexes in substrates involved in Aβ removal, including TREM2, RAGE and APOER2. Current work is expanding on the specific role of presenilin-1 and presenilin-2 on the regulation of Aβ degrading enzymes, and microglial Aβ removal.

Conclusions: Our findings to date indicate that presenilin-2 has a more important role in Aβ metabolism than previously considered. This study sheds new light on the complexity of presenilin biology in the context of Alzheimer’s disease and elucidates their underappreciated roles in Aβ removal.
SUPER-RESOLUTION STUDIES OF ABETA42 AND ITS PRECURSORS IN NEURONS

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**Aims:** Our aim is to – at a cellular level – further understand the generation and polymerization of Aβ and its role in AD pathogenesis. Our approach to achieve this aim is to study how Aβ is formed from its precursor APP and explore the different pools of Aβ in subcellular details in neurons by using super-resolution microscopy.

**Methods:** We used advanced fluorescence microscopes to resolve the subcellular details of neurons, making it possible to visualize even single synaptic vesicles. Mouse primary hippocampal neurons were immunolabelled and imaged by stimulated emission depletion (STED) microscopy, including three-dimensional three-channel imaging, and quantitative image analyses. We also used live cell imaging to study Aβ uptake and transport.

**Results:** We showed that N- and C-terminal fragments of APP (APP-CTFs) are sorted in early endosomes in soma, and that Aβ42, APP-CTFs and gamma-secretase are all enriched in the presynapse, suggesting that the presynapse is a site of Aβ42 production. In contrast, exogenous Aβ42 was enriched at the plasma membrane of the neuron, endocytosed and trafficked to late endosomes and lysosomes, and eventually accumulated in the soma.

**Conclusions:** Super-resolution microscopy is a valuable technique for studying Aβ and its precursors at a subcellular level. The presynapse appears to be an important site for Aβ generation, while endocytosed Aβ is transported to somatic endosomal/lysosomal compartments. Further investigations are necessary to characterize the physiological/pathological roles of Aβ in the different pools.
Aims: We investigate which of the hypotheses that explain the selective vulnerability of dopaminergic neurons (DNs) in substantia nigra pars compacta (SN) tissue in Parkinson’s disease (PD) are supported on a snRNA-seq dataset of 13 controls and 14 cases of 79(73-85) years (18 males, 9 females), by using gene co-expression networks (GCNs).

Methods: For each cell type subcluster, we propose reducing gene expression sparsity creating pseudo-cells while the number of cells is > 200, thus getting multiple matrices and creating multiple GCNs using CoExpNets package.

Results: Our models support the hypothesis of axonal transport dysregulation in PD through a strong association with diagnosis (P<5.97E-26) and enrichment of GO terms like microtubule-based process (P<6.85E-7) and axonal transport (P<1.66E-6) and expression enrichment for SN markers (P<9.53E-05). They evidence an association with age (P<1.81E-06) too but found that axonal transport’s association is exclusive for diagnosis, with no causal connection with age. The same models support the hypothesis of iron storage dysregulation in PD because FTL, FTH1 and GPX4 are relevant genes in a reliable gene cluster (98%, 100% and 100% percentile in module membership, respectively). It is associated with diagnosis (P<2.18E-56) and age (P<2.52E-61), but FTL was exclusively associated with diagnosis, therefore, iron storage dysregulation is not associated with aging. GPX4 is down-regulated in PD cases (P<4.65E-13, -0.18 log2FC), suggesting that ferroptosis is not controlled well.

Conclusions: Our study supports the hypotheses of massive axonal arborization and iron accumulation in PD and opens new avenues of research, i.e. finding evidence to the convergence of theses hypotheses.
APP ACCUMULATES WITH PRESYNAPTIC PROTEINS AROUND AMYLOID PLAQUES: A ROLE FOR PRESYNAPTIC MECHANISMS IN ALZHEIMER’S DISEASE?

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**Aims:** In Alzheimer’s disease (AD), the distribution of the amyloid precursor protein (APP) and its fragments other than A\(\beta\), has not been fully characterized.

**Methods:** Here, we investigate the distribution of APP and its fragments in human AD brain samples and in mouse models of AD in reference to its proteases, synaptic proteins, and histopathological features characteristic of AD brain, by combining an extensive set of histological and analytical tools.

**Results:** We report that the prominent somatic distribution of APP observed in control patients remarkably vanishes in human AD patients to the benefit of dense accumulations of extra-somatic APP, which surround dense-core amyloid plaques enriched in APP-Nter. These features are accentuated in patients with familial forms of the disease. Importantly, APP accumulations are enriched in phosphorylated-Tau and presynaptic proteins whereas they are depleted of post-synaptic proteins suggesting that the extra-somatic accumulations of APP are of presynaptic origin. Moreover, BACE1 and PS1, the proteases respectively responsible for APP ectodomain shedding and intramembrane proteolysis are enriched within APP accumulations, suggesting that APP could be cleaved in the accumulations, and supply adjacent amyloid plaques with APP-Nter and A\(\beta\) peptides. Ultrastructural analyses unveil that APP concentrates in autophagosomes and in multivesicular bodies together with presynaptic vesicle proteins.

**Conclusions:** Altogether, alteration of APP distribution and its accumulation together with presynaptic proteins around dense-core amyloid plaques is a key histopathological feature in AD, lending support to the notion that presynaptic failure is a strong physiopathological component of AD.
INFLUENCE OF THE DOMINANTLY AFFECTED SIDE ON MORPHOLOGICAL CORRELATES OF MOTOR RESERVE IN PARKINSON'S DISEASE

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Aims: One of the hallmarks of Parkinson’s Disease (PD) is the significant asymmetry of motor symptoms and the underlying striatal dopaminergic terminal loss. Motor reserve (MR) is an important factor mitigating the effect of neurodegeneration on motor symptoms. Here, we explored whether the structural correlates of MR may depend on the clinically more affected body side.

Methods: Data of 151 PD patients with MRI, DaT-SPECT and clinical information were included from the PPMI database (ppmi-info.org). Using the residual approach, we determined MR-residuals by means of putaminal dopamine signal and motor symptom severity. Next, we grouped individuals into a high (n=50, 20 left-affected, 30 right-affected) and low (n=44, 22 left-affected, 22 right-affected) MR group by median split of the MR-residuals. The influence of the more affected body side on grey matter volume (GMV) maps (extracted with CAT12) across MR groups was assessed using a full-factorial ANCOVA in SPM12 (k>100 voxel, FWE corrected).

Results: The high MR group presented greater GMV in the left postcentral and left occipital fusiform gyrus as compared to the low MR group. A group by side interaction was observed only for patients with right dominance. Right-dominantly affected high MR individuals presented greater GMV in right inferior frontal and left superior temporal gyrus. No differences were observed for left-dominantly affected patients.

Conclusions: Higher MR seems to be associated with greater GMV in the left hemisphere irrespective of the more affected body side. Moreover, we observed an additional involvement of left- and right-sided brain areas when the right body side was more affected.
ECTO-GPR37: A POTENTIAL BIOMARKER FOR PARKINSON’S DISEASE

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Aims: There is an urgent need to find improved diagnostic and prognostic biomarkers reflecting pathological progression of Parkinson’s disease (PD). GPR37 is an orphan G protein-coupled receptor that toxically accumulates in autosomal recessive juvenile parkinsonism. Here, we investigated whether GPR37 is upregulated in sporadic PD, and thus a potential candidate biomarker for PD.

Methods: GPR37 protein density and mRNA expression in postmortem substantia nigra (SN) from PD patients were analysed by immunoblot and RT-qPCR, respectively. The presence of peptides from the N-terminus-cleaved domain of GPR37 (i.e. ecto-GPR37) in human cerebrospinal fluid (CSF) was determined by liquid chromatography-mass spectrometric analysis. An engineered in-house nanoluciferase-based immunoassay was used to quantify ecto-GPR37 in CSF samples from GPR37+/+ and GPR37-/-, mice to validate the specificity of the technique. Next, neurological control subjects, PD patients and Alzheimer’s disease (AD) patients.

Results: GPR37 protein density and mRNA expression were significantly augmented in sporadic PD. Increased amounts of ecto-GPR37 peptides in the CSF samples from PD patients were identified by mass spectrometry and quantified by the in-house ELISA method. However, the CSF total α-synuclein level in PD patients did not differ from that in NC subjects. Similarly, the cortical GPR37 mRNA expression and CSF ecto-GPR37 levels in AD patients were also unaltered.

Conclusions: GPR37 expression is increased in SN of sporadic PD patients. The ecto-GPR37 peptides are significantly increased in the CSF of PD patients, but not in AD patients. These results open perspectives and encourage further clinical studies to confirm the validity and utility of ecto-GPR37 as a potential PD biomarker.
GLYCATION MODULATES GLUTAMERGIC SIGNALING AND EXACERBATES PARKINSON’S DISEASE-LIKE PHENOTYPERS

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Aims: Alpha-synuclein (aSyn) is a central player in the pathogenesis of synucleinopathies due to its accumulation in typical protein aggregates in the brain. However, it is still unclear how it contributes to neurodegeneration. Type-2 diabetes mellitus is a risk factor for Parkinson’s disease (PD) and, one common molecular alteration among these disorders is an age-associated increase in protein glycation. We hypothesized that glycation-induced dysfunction of neuronal pathways might be an underlying molecular cause of synucleinopathies. Here, we dissected the specific impact of methylglyoxal (MGO, a glycating agent) in mice overexpressing aSyn in the brain.

Methods: Age-matched (16 weeks old) male aSyn transgenic (Thy1-aSyn) or WT mice received a single dose of MGO or vehicle via intracerebroventricular (ICV) injection.

Results: We found that glycation potentiates motor, cognitive, olfactory, and colonic dysfunction. aSyn accumulates in the midbrain, striatum, and prefrontal cortex, and protein glycation is increased in the cerebellum and midbrain, where neuronal and dopaminergic cell loss is detected. Quantitative proteomic analysis revealed that MGO mainly impacts on glutamatergic proteins in the midbrain (NMDA, AMPA, glutaminase, VGLUT and EAAT1), but not in the prefrontal cortex, where it mainly affects the electron transport chain.
Conclusions: Overall, we demonstrated that MGO-induced glycation accelerates PD-like sensorimotor and cognitive alterations and suggest that the increase of glutamatergic signaling may represent a compensatory mechanism to the MGO-induced dopaminergic neurodegeneration. Our study sheds light into the enhanced vulnerability of the midbrain in Parkinson’s disease-related synaptic dysfunction, and suggests that glycation suppressors and anti-glutamatergic drugs hold promise as disease-modifying therapies for synucleinopathies.
ON-DEMAND SYMPOSIUM: PD, LBD MECHANISMS, DIAGNOSIS, BIOMARKERS, IMAGING

HEME OXYGENASE-1, DOWNSTREAM MICRORNA EXPRESSION AND PARKINSON’S DISEASE

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Aims: Dysregulated microRNAs (miRNAs) play a major role in various neurodegenerative conditions, including Parkinson’s disease (PD). Heme oxygenase-1 (HO-1) has been implicated in PD pathology, and we previously showed that altered expression profiles of salient miRNAs and their mRNA targets contribute to neural damage accruing from the overexpression of glial HO-1. Two miRNAs, miR-153 and miR-223, were identified as regulators of alpha-synuclein acting downstream of HO-1. Aims: To assess the diagnostic potential of miR-153 and miR-223 in mice and humans with parkinsonism, and elucidate the potential mechanism that links peripheral and neurological pathologies in neurodegenerative disease.

Methods: Parkinsonian transgenic (TG) GFAP.HMOX1 mice, engineered to overexpress the human HO-1 gene (HMOX1) in astrocytes between 8.5 and 19 months of age, were evaluated. MiRNA levels were measured by RT-qPCR in mice and human saliva. Extracellular vesicle (EV) content of human saliva was assayed using polymer- and immunoaffinity-based isolation methods, followed by nano flow liquid chromatography-tandem mass spectrometry.

Results: Downstream of HO-1 overexpression, miR-153 and miR-223 were significantly downregulated in TG mice basal ganglia and serum compared to wild-type controls. Moreover, miR-153 and miR-223 were similarly decreased in the saliva of human PD subjects (n=83) compared to healthy controls (n=77). Further analysis of EVs from PD saliva (n=4) via mass spectrometry revealed alterations in key proteins relating to PD (DJ-1) and oxidative stress (SOD2, HSPB1) compared to healthy controls (n=4).

Conclusions: HO-1-mediated (and potentially EV HO-1-mediated) perturbations in brain and peripheral miRNA expression profiles may drive PD neuropathology and serve as diagnostic markers for this condition.
ON-DEMAND SYMPOSIUM: PD, LBD MECHANISMS, DIAGNOSIS, BIOMARKERS, IMAGING

DYNACTIN P150GLUED–DEFICIENCY IN MIDBRAIN DOPAMINERGIC NEURONS LEADS TO PROGRESSIVE NEURODEGENERATION AND ENDOPLASMIC RETICULUM DYSFUNCTION

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Aims: Missense mutations in DCTN1 were linked to Perry syndrome (PS), a rare neurodegenerative disease clinically manifested with parkinsonism and mental depression. Here we try to understand how the dysfunction of DCTN1-encoded dynactin p150Glued protein contributes to PS-related dopaminergic neuron (DAN) loss.

Methods: We crossbred a line of Dctn1 knock-in (KI) mice with Th-Cre or Cre/Esr1 mice to generate the bigenic Dctn1 conditional knockout (cKO) mice for genetic deletion of p150Glued protein in midbrain DANs or other cells. We then performed series of biochemical, neurochemical, cell biology, behavioral and neuropathological studies on the cKO mice or cells to investigate the underlying pathological mechanisms.

Results: Genetic deletion of p150Glued in midbrain DANs led to progressive motor impairments, degeneration of midbrain DANs, axon atrophy and astrogliosis. The aged cKO mice showed increased accumulation of α-synuclein in the soma and nucleus of midbrain DANs. The axons and dendrites of cKO DANs also contained unusually large sphere structures. Further studies revealed abnormal accumulation of endoplasmic reticulum (ER) residential protein BiP in the dendritic spheroids and soma of cKO DANs. Accordingly, the Dctn1 cKO cells displayed reduced levels of COPII cargo proteins critical for ER export, increased unfolded protein response (UPR), and more vulnerability to ER stress-induced cell death.

Conclusions: Our findings demonstrate that p150Glued is important in maintaining the function and survival of midbrain DANs during aging. We particularly highlighted the role of p150Glued in stabilizing the molecular machinery for ER export, suggesting that the PS-related p150Glued deficiency may render the midbrain DANs more susceptible to UPR and ER stress.
DEVELOPING A CORE OUTCOME SET FOR LEWY BODY DEMENTIA

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**Aims:** Lewy body dementia (LBD) is an important dementia cause including motor, neuropsychiatric and autonomic manifestations. Compared to, e.g., Alzheimer, LBD has significantly fewer intervention studies, often with diverse outcome measures, making comparison and clinical implementation difficult. A Core Outcome Set (COS) can address this by ensuring that data are comparable, relevant, useful, and usable for making the best healthcare decisions. Thus, the aim was to develop an LBD-specific COS suitable for (non-)pharmacological trials (https://www.comet-initiative.org/Studies/Details/1963).

**Methods:** Using a multi-stage approach, we first undertook a narrative review of LBD intervention studies to inform a more rigorous systematic review. Using these study outcomes, we created an outcome list that were subjected to an e-Delphi consensus process to create an outcome shortlist. Input was sought from stakeholders with lived LBD experience and clinicians, scientists, policy and third sector leads. Finally, through a consensus meeting, we selected appropriate instruments to measure the COS construct outcomes for use in research and clinical practice.

**Results:** The COS applies to LBD people and their care partners including assessments of motor, cognitive, neuropsychiatric, autonomic, health economic, health provision, and quality of life outcomes. Baseline demographic and clinical variables as well as descriptors are included to enable case-mix adjustment. Measurement instruments aligned to each outcome domain are suggested. The COS is now ready for use and pilot testing.

**Conclusions:** This provides the first LBD-specific COS to standardly document, report, and compare LBD-related outcomes, providing an international benchmark. This will improve our understanding of the disease course and foster informed and cost-effective healthcare decisions.
Aims: Visual complaints can have a vast impact on the quality of life of people with Parkinson’s disease (pwPD). In clinical practice, however, visual complaints often go undetected. A better understanding of visual complaints is necessary to optimize care for pwPD and visual complaints. This study aims at determining the prevalence of visual complaints experienced by an outpatient cohort of pwPD compared to a control group. In addition, relations between visual complaints and demographic and disease related variables are investigated.

Methods: The Screening of Visual Complaints questionnaire (SVCq) screened for 19 visual complaints in a large cohort of pwPD (n = 581) and an age-matched control group (n = 583).

Results: PwPD experienced significantly more complaints (‘often/always’; 2.07% to 21.69%) than control subjects (0.34% to 10.98%). In addition, they experienced more limitations in daily life due to visual complaints. Most common were complaints regarding reading, unclear vision, trouble focusing, reduced contrast, blinded by bright light, and needing more light. The visual complaints in pwPD could only partially be explained by the presence of ophthalmological conditions and are likely to also result from factors directly or indirectly related to the disease itself. Age, disease duration, and disease severity had a significant positive relationship with the prevalence and severity of visual complaints in pwPD. Most complaints did not differ between the sexes.

Conclusions: Visual complaints are highly prevalent in pwPD and have a vast impact on their daily lives. Active questioning is advised for timely recognition and treatment of these complaints.
VALIDITY AND RELIABILITY OF A COMPOSITE COGNITIVE OUTCOME MEASURE FOR CLINICAL TRIALS IN DEMENTIA WITH LEWY BODIES

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Aims: Cognitive assessments may be used to generate different kinds of evidence during clinical drug development including use in pharmacodynamic/early signal detection, safety/tolerability, and primary, co-primary, secondary, and exploratory efficacy contexts. When used to generate evidence of treatment benefit, multicomponent cognition assessments may be viewed as performance outcome (PerfO) type clinical outcome assessments (COAs) for which evidence of validity, reliability, and sensitivity to change should be established.

Methods: As part of a Phase 2a clinical trial in dementia with Lewy bodies (DLB), 115 patients were screened for possible inclusion with Clinical Dementia Rating (CDR), MMSE, and cognitive test battery assessments. A composite outcome measure was derived from the cognitive test battery. We present here preliminary validity and reliability data based on screening and baseline data.

Results: The composite showed an expected correlation with both MMSE total score (r=0.67) and CDR-SB (r=0.51), supporting construct validity. Additionally, scores for the composite were statistically significantly lower (p<0.001) for patients with CDR-Global scores indicating dementia ≥1 (N=49; mean -0.22; SD 0.72) versus those with CDR-Global scores of ≤0.5 (N=29; mean 0.36; SD 0.695), supporting known groups validity. Test-retest reliability across the screening 1 and baseline visits was good (ICC=0.86).

Conclusions: These initial data suggest a composite comprised of assessments of reaction time, attention, visual learning, working memory and verbal fluency are a valid and reliable assessment of cognition in DLB clinical trials.
ON-DEMAND SYMPOSIUM: PD, LBD MECHANISMS, DIAGNOSIS, BIOMARKERS, IMAGING

NEUROPSYCHIATRIC SYMPTOMS INFLUENCE COGNITION DIFFERENTLY PARKINSON'S DISEASE WITH AND WITHOUT DOPAMINERGIC DEFICIT.

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Aims: In Parkinson's disease (PD), various symptoms can occur before the onset of motor symptoms and increase the risk of cognitive decline such as neuropsychiatric symptoms (NPS). We propose to understand whether NPS can modify PD biomarkers and cognitive performance depending on clinical status.

Methods: The PPMI database was used to analyze 121 Healthy Control (HC), 241 PD with dopaminergic deficit (PDwDD) and 35 PD without dopaminergic deficit (PDnoDD, as defined by reduced striatal dopamine). Neuropsychiatric, neuropsychological and striatal dopamine levels (based on DAT scan data) were extracted. We performed a MANCOVA model with 3 clinical groups and 4 NPS (hallucinations, depression, anxiety, apathy) as fixed factors, and cognitive, striatal dopamine levels as dependent variables, covaried by age, education, sex and ethnicity.

Results: Simple group effects showed that PDwDD had lower working memory (WM) and semantic fluency performance in comparison to HC and PDnoDD. In addition, depression, anxiety and apathy were associated with lower cognitive performance. In the group-NPS interactions, hallucinations were shown to improve WM in PDwDD, depression reduced WM in PDnoDD, and anxiety reduced semantic fluency in PDnoDD. No effects were depicted on the striatal dopamine levels.

Conclusions: Our results suggest a role of NPS on cognitive performance in both PDwDD and PDnoDD but with distinct domains affected. Yet, this distinction doesn’t seem to be confirmed by a potential effect on striatal dopamine levels that are used for the PDwDD classification.
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Aims: Autonomic nervous system pathology manifests early in Parkinson’s disease (PD) course. Although heart rate variability measured by a 5-minute electrocardiogram (ECG) is reported to be reduced in PD, little is known about ECG markers during prodromal disease, and brief 10-second ECGs have been rarely studied.

Methods: We obtained data for PD and date/age/sex/race-matched control subjects from electronic health records of Loyola University Chicago (LUC) and University of Tennessee-Methodist LeBonheur Healthcare (MLH). We used signal processing and deep machine learning to predict PD risk from standard 10-second 12-lead ECGs performed between 6 months-5 years before diagnosis. The prediction model was built with MLH data and externally validated with LUC data.

Results: We identified 129 cases/1058 controls at MLH and 47 cases/165 controls at LUC. We initially trained models on 90% of MLH data and internally validated them in the remaining 10%. The best performing model with an internal validation area under the curve (AUC) of 0.73 was a one-dimensional convolutional neural networks model. We further externally validated this using LUC data that yielded an AUC of 0.67. When we considered only ECGs obtained 6 months to 1-year preceding PD diagnosis, the external validation AUC was 0.74.

Conclusions: Using only a simple 10-second ECG we built a predictive model that correctly classified individuals with prodromal PD with modest accuracy. The model was effective in an independent cohort, particularly closer to disease diagnosis. Standard ECGs may help to identify individuals with prodromal PD for inclusion in disease-modifying therapeutic trials.
ON-DEMAND SYMPOSIUM: PD, LBD MECHANISMS, DIAGNOSIS, BIOMARKERS, IMAGING

ADDENBROOKE’S COGNITIVE EXAMINATION III (ACE-III): DIAGNOSTIC UTILITY FOR DETECTING MILD COGNITIVE IMPAIRMENT AND DEMENTIA IN PARKINSON’S DISEASE

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Aims: To investigate the diagnostic accuracy, sensitivity, and specificity of the Addenbrooke’s Cognitive Examination-III in patients with Parkinson’s disease (PD), using the comprehensive neuropsychological battery as reference method.

Methods: Cross-sectional, observational, case-control study. Setting: rehabilitation service. 150 patients and 60 healthy controls matched for age, sex, and education. The individuals were evaluated individually in two sessions of approximately 90 minutes each. For level I assessment, ACE-III was used. Level II assessment used a comprehensive neuropsychological battery of standardized tests for this population. All patients remained in on-state during the study. The diagnostic accuracy of the battery was investigated through the analysis of the ROC (Receiver Operating Characteristic).

Results: The clinical group was divided into three subgroups: normal cognition in Parkinson’s disease (NC-PD-16%), mild cognitive impairment due to Parkinson's disease (MCI-PD-69.33%), and dementia due to Parkinson's disease (DPD-14.66%). ACE-III optimal cut-off scores for detecting MCI-PD and DPD were 85/100 (sensitivity 58.65%, specificity 60%) and 81/100 points (sensitivity 77.27%, specificity 78.33%), respectively. Age was inversely associated with the performance of the scores (totals and domains of the ACE-III), while the level of education had a significantly positive correlation in the performance of these scores. There was a significant correlation with standardized neuropsychological tests for this population.

Conclusions: ACE-III is a useful test for assessing the cognitive domains in PD and differentiates individuals with MCI-PD and DPD from healthy controls. Future research, in a community setting, is necessary to provide discriminatory capacity of ACE-III in the different severities of dementia.
ON-DEMAND SYMPOSIUM: TAUPATHIES: MECHANISMS & TREATMENT STRATEGIES 1

EXPLORING THE THERAPEUTIC OPTIONS OF A LIPOLYTIC SIGNALING PATHWAY IN THE TREATMENT OF COGNITIVE DECLINE ASSOCIATED WITH NEURODEGENERATIVE STATES

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Aims: Phospholipase D (PLD), a lipolytic enzyme for membrane phospholipids, exists in two isoforms of mammalian phosphatidyl choline (PC) specific PLD, inducible PC-PLD1 and constitutive PC-PLD2. Elevated PLD activity is well documented in clinical studies involving Alzheimer’s disease (AD). Our lab was the first to show elevated PLD1 facilitates synaptic dysfunction and underlying memory deficits driven by amyloidogenic insults by Aβ/tau. In this presentation, we will demonstrate the aberrant overexpression of PLD isoform expression, activity and signaling in the progression of the cognitive decline associated with neurodegenerative states including Alzheimer’s Disease and Frontotemporal Dementia. Additionally, we will provide proof-of-concept in the use of small molecule inhibitors that are well-tolerated in the prevention of cognitive decline using different corroborative approaches (functional and observational) in human clinical samples and transgenic animal models.

Methods: Human clinical samples were assessed for functional (FASS-LTP) and pathological (immunofluorescence) effects of PLD1 overexpression. Transgenic rodent models (Tg-AD, 3xTg-AD) were used for functional verification of therapeutic potential and mechanism of action of potential small molecule inhibitors in preventing cognitive decline at different age groups and sex-specific changes.

Results: A clear change of PLD1 signalosome actions were observed in the synaptic dysfunction underlying the cognitive decline, as established in human clinical conditions and corroborated in transgenic rodent models of ADRD.

Conclusions: Targeting PLD1 signalosome may be a great complementary treatment to immunosuppressive therapeutic approaches in preserving cognitive states in ADRD patients.
ON-DEMAND SYMPOSIUM: TAUPATHIES: MECHANISMS & TREATMENT STRATEGIES 1

SAUNA-LIKE CONDITIONS OR MENTHOL TREATMENT REDUCE TAU PHOSPHORYLATION THROUGH MILD HYPERTERMIA

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Aims: In Alzheimer's disease (AD), hyper-phosphorylation and aggregation of tau correlates with clinical progression and represents a valid therapeutic target. A recent 20-year prospective study by Laukkanen et al. revealed an association between moderate to high frequency of Finnish sauna bathing and a lower incidence of dementia and AD, but the molecular mechanisms underlying these benefits remain uncertain. Interestingly, a consequence of sauna is to induce mild hyperthermia, as 20 min is sufficient to raise body temperature to approximately 38-39°C. Here, we tested the hypothesis that sauna-like conditions could lower tau phosphorylation by increasing body temperature.

Methods: In a first set of experiments, we investigated the effect of mild hyperthermia (38-39°C) on the phosphorylation of tau in both neuronal-like cells (SH, N2A) and mice (wild-type and hTau) and analyzed tau phosphorylation. We then explored druggable ways to promote thermogenesis and increase body temperature. We selected menthol, as it triggers the cold-sensitive TRPM8 receptors (Transient receptor potential cation channel subfamily M member 8) and promotes thermogenesis.

Results: Higher temperatures decreased tau phosphorylation in both neuronal-like cells (SH, N2A) and mice (wild-type and hTau), but we could not detect changes in insoluble tau. Topical application of menthol led to a significant and sustained increase (hours) in rectal temperature of hTau mice (+1.3°C) and resulted in a significant decrease in phosphorylation of tau.

Conclusions: Our results suggest that sauna-like conditions and menthol treatment can lower tau pathology through mild hyperthermia and may explain the beneficial effects of sauna bathing. These findings open new potential therapeutic treatments.
PNT001, A SELECTIVE CIS-PT231 TAU ANTIBODY, TARGETS BRAIN TAU SEEDS IN ALZHEIMER’S DISEASE, PROGRESSIVE SUPRANUCLEAR PALSY, AND A MOUSE MODEL OF TAUOPATHY

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Aims: cis-pT231 (cistau) has been proposed as an early pathogenic and seed-competent form of tau in tauopathies. Here, we use ultrasensitive assays for tau seeding activities to assess if a selective human cistau antibody, PNT001, can target brain tau seeds in Alzheimer’s disease (AD), progressive supranuclear palsy (PSP), and a mouse model of tauopathy.

Methods: Brain tissue obtained from neuropathologically confirmed AD (n=2) and PSP (n=5) cases was homogenized and subjected to immunodepletions with PNT001, AT8, antibodies to N-terminal and mid-domain tau epitopes, or isotype control antibodies and used for subsequent assessment of tau seeding activities. Tissue from a mouse model of tauopathy, Tg4510, was also analyzed. Tau seeding activities were measured using the ultrasensitive seed amplification assays [real-time quaking induced conversion (RT-QuIC)] for selective detection of 4R and 3R/4R tau seeds.

Results: Immunodepletions using PNT001 reduced seeding activities in AD hippocampus and frontal cortex tissue on average by 89 and 82%, respectively, and in PSP superior frontal gyrus by 90%. Seed depletion with other antibodies including AT8 was less efficient, with seed depletions of 62% and 52% for AD frontal cortex and hippocampus, and 71% for PSP. PNT001 was also able to target tau proteoforms in a mouse model of tauopathy (Tg4510), reducing seeding activity by 65% whereas AT8 depleted seeding activities by 22%.

Conclusions: PNT001 targets tau seeds that occur in both 3R/4R and 4R tauopathies of AD and PSP. This further indicates cistau is included in seed-competent forms of tau that can be targeted with antibody therapies.
Aims: Aqueously soluble oligomers of the Aβ protein, rather than monomers or insoluble fibrils, are considered the most bioactive form. Although extensive characterization of synthetic oligomers has been performed, little is known about the structure of oligomers that occur in human brain; instead, they are operationally defined as that non-monomeric material present in the supernatant after ultracentrifugation of an aqueously soluble extract.

Methods: Soluble extracts were prepared in TBS using homogenization or soaking, followed by ultracentrifugation in the SW41Ti rotor at 200,000 g. Re-pelleting was performed in a tabletop centrifuge at 20,000 g. Immunogold labeling used D54D2 (rabbit anti-Aβ N-terminus) or various rabbit anti-phosphotau monoclonal antibodies (Cell Signaling Technologies). ELISA utilized m266 and 21F12 after denaturing samples in 5M guanidine hydrochloride.

Results: We found that short filamentous structures could be re-pelleted from soluble extracts of AD but not control cortex in a concentration-dependent manner, and visualized by negative-stain electron microscopy. Immunogold labeling confirmed the presence of Aβ. Re-pelleting of Aβ filaments from soluble post-200,000 g extracts was enhanced by freezing at least overnight and by adding Triton X-100, Tween-20, and digitonin, but not CHAPSO, but was prevented by high ionic strength. Linear time-dependence in re-pelleting suggested the filaments were not reconstituted during re-pelleting and may be present in the initial post-200,000 g aqueous extract. We also observed paired helical filaments re-pelleting from aqueous extracts labeled by anti-phosphotau immunogold.

Conclusions: These results suggest that at least some high molecular weight oligomers of Aβ and tau in putatively soluble fractions are, and/or become, filamentous.
ALZHEIMER PHF-TAU DO NOT SPREAD TAU PATHOLOGY TO THE BRAIN VIA THE RETINOTECTAL PATHWAY AFTER INTRAOCULAR INJECTION IN MOUSE MODELS


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Aims: The formation of neurofibrillary tangles found in Alzheimer’s disease (AD) followed neuroanatomical pathways suggesting that synaptically connected neurons could transmit tau pathology by the recruitment of normal tau in a prion like manner. Moreover, the intracerebral injection of pathological tau from AD brains induces the seeding of normal tau in mouse brain. Scrapie agent can spread across the retinotectal pathway after intraocular injection of scrapie mouse brain homogenates. CJD has been transmitted after ocular transplants. In AD, tau pathology was detected in the retina. We investigated the potential risk of tau pathology transmission to the brain after an eye surgery.

Methods: We have analysed the formation of tau pathology along the visual pathway in the geniculate nucleus and in the superior colliculus of WT, hTau and Tg22 mice after intraocular injection of PHF-tau proteins from AD brains (or CTL fraction from healthy brains).

Results: 6 hours after the injection, PHF-tau proteins are internalized by retinal ganglion cells of AD injected mice. Tau pathology was never observed in the geniculate nucleus of WT, hTau or Tg22 mice. In the superior colliculus of Tg22 mice, a tau pathology was detected with PHF-1, AT8 and MC1 antibodies but the quantifications of tau pathology do not show any differences between AD or CTL injected mice.

Conclusions: These results suggest that ocular transplants from subjects with tau pathology would present little risk.
INCREASED TAU PROTEIN SECRETION VIA EXOSOMES IN NEURONS AGING IN VITRO BY NPC1-MEDIATED ENDOosomal CHOLESTEROL ACCUMULATION

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Aims: In Alzheimer’s disease (AD) patients, neurofibrillary tangle (NFT) pathology appears to spread throughout the brain and this correlates with the symptoms. Exosomes, a type of small extracellular vesicles (EVs), have been proposed as one of the underlying mechanisms by which aggregated tau passes from neuron to neuron. Exosomes originate in multivesicular bodies (MVBs), a subtype of late-endosomes, in the form of intraluminal vesicles (ILVs) that contain cytosolic proteins. After MVBs fuse to the plasma membrane, ILVs are released to the extracellular space in form of exosomes. A recent study found that tau protein spreads twice as fast in older mice as compared to younger animals (Wegmann et al., Sci Adv., 2019). In this work, we set out to finding out the mechanisms by which aging speeds up the transmission of tau.

Methods: We investigated the aging-related molecular alterations leading to enhanced tau spread in neurons aging in vitro.

Results: We show that old neurons in vitro secrete higher amounts of total tau via exosomes by a mechanism involving the increased generation of ILVs. We also show that the high number of ILVs results from the accumulation of cholesterol in MVBs, which in turn is due to decreased levels of the cholesterol extruding protein NPC1. NPC1 down-regulation results from the upregulation of the NPC1 repressor microRNA 33, combined with the Akt-mTOR-mediated NPC1 degradation by the proteasome.

Conclusions: Although releasing more exosomes can help old neurons to eliminate waste material, it may contribute to the spread of pathological forms of tau.
ON-DEMAND SYMPOSIUM: TAUPATHIES: MECHANISMS & TREATMENT STRATEGIES 1

DIGITAL QUANTIFICATION OF CELLULAR-LEVEL TAU PATHOLOGY USING NUCLEI-DETECTION-BASED IMAGE ANALYSIS IN PROGRESSIVE SUPRANUCLEAR PALSY

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Aims: To elucidate novel patterns of cellular pathology underlying neurodegenerative diseases we developed a pipeline for automated whole-slide image analyses.

Methods: We analysed 14 systematically sampled regions from 36 brains donated by patients with a clinical and pathological diagnosis of progressive supranuclear palsy (PSP). Sections were stained for hyperphosphorylated tau (AT8) and scanned at 40x (Aperio AT2, Leica). The image analysis pipeline was generated using the StarDist plugin within QuPath. Precision and correlation to manual cell detections and pathology scores were tested by Jaccard index and Spearman correlation. Effects of region, sex and age on the numbers of AT8-positive cells, and the proportion to total tau-burden (AT8-positive pixels), were modelled using Bayesian linear mixed effect models (brms, R).

Results: Our methodology showed a high precision for cell nuclei detection (Jaccard index=0.87: 10 fields of view; 3571 nuclei) and a tight correlation to manual pathology scores within regions ($r=0.82$, $p<0.05$). Modelling the regional cell-level pathology we found $>1:100$ AT8-positive cells in subthalamic nucleus, midbrain, globus pallidus and premotor cortex; and $<1:1000$ AT8-positive cells in the temporal and occipital cortex. We found no effect of age or sex (BayesFactor<0.3). The pixels associated with AT8-positive cells corresponded to 36.8% of all AT8-positive pixels, but within regions there was a negative relationship between cell-soma and non-soma AT8-positive pixels (BayesFactor>100).

Conclusions: We have developed and validated a deep-learning tool to detect and quantify cellular-level pathology and applied it in the primary tauopathy PSP. Our results suggest that tau-aggregates is greater is faster around the soma than as neuropil threads.
ON-DEMAND SYMPOSIUM: TAUPATHIES: MECHANISMS & TREATMENT STRATEGIES

SAFETY, PHARMACOKINETICS, AND PHARMACODYNAMICS OF SINGLE AND MULTIPLE ASCENDING DOSES OF THE NOVEL ANTI-TAU THERAPEUTIC ANTIBODY E2814: A PHASE 1, FIRST-IN-HUMAN STUDY IN HEALTHY VOLUNTEERS

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Aims: E2814 is a novel anti-tau therapeutic monoclonal antibody (mAb) that inhibits the propagation of pathological tau species by binding to the microtubule binding region (MTBR). This study evaluated safety, pharmacokinetics (PK), immunogenicity and target engagement (TE) of single and multiple doses of E2814.

Methods: This randomized, placebo-controlled, Phase 1 study evaluated 3 single ascending doses (SAD) and 2 multiple ascending doses (MAD) in healthy adults. Subjects (n=8/cohort) received single or multiple (every 4 weeks) 1-hour IV infusions of E2814 (or placebo). PK was characterized in blood and CSF samples. TE was evaluated in CSF by measuring E2814-bound and free MTBR-tau proxy peptide concentrations (MTBR-tau354 and MTBR-tau299 containing epitopes in R4 and R2, respectively).

Results: E2814 has an adequate single and multiple dose safety profile. No dose limiting events were observed. E2814 exposures (Cmax, AUC) increased in a dose-related manner. E2814 accumulation index on multiple dosing (Racc=1.5) was consistent with its elimination half-life (22 days). The serum-to-CSF ratio was 0.2%. TE was dose related and sustained over time with maximum TE levels of 45% and 76% in the SAD and 47% and 62% in the MAD for MTBR-tau354 and MTBR-tau299, respectively. In the SAD, 2 out of 24 subjects had transient low level anti-drug antibody (ADA) titers by the last study day; no post dose ADA were detected in the MAD.

Conclusions: E2814 presented an adequate safety, PK and immunogenicity profile in healthy adults. Increase in TE was dose-related and sustained. These results support further development of E2814 as a disease-modifying therapy for AD.
THE ANTI-AMYLOIDOGENIC ACTIVITY OF CAFFEIC ACID IN A NEURONAL MEMBRANE-LIKE ENVIRONMENT: RELEVANCE FOR THE ALZHEIMER'S DISEASE

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Aims: This work aims to evaluate the ability of caffeic acid (CA) in inhibiting the Aβ\textsubscript{1-42} aggregation and disrupting mature fibrils in a neuronal membrane-like environment.

Methods: Aβ\textsubscript{1-42} monomers or fibrils were treated with CA in the presence or absence of in vitro models of neuronal membranes mimicked by liposomes. The samples were incubated at 37 °C for 4 h with continuous medium agitation. The content of amyloid fibrils upon incubation of the peptide with CA was quantified through a thioflavin T (ThT) fluorescence assay.

Results: No significant difference was detected in the ThT signal at the beginning and end of the experiments in the aqueous environment, suggesting that CA fully prevents the fibrillation process. In addition, the overall CA’s ability in preventing the fibril formation was not disturbed by the lipid membranes. Concerning the disaggregation experiments, the presence of CA immediately suppressed around 75% of the ThT fluorescence intensity in the aqueous medium, suggesting fibril disruption. However, the presence of the lipid membranes significantly affected the CA’s ability in disrupting mature fibrils. Instead of being immediate, the disaggregating activity of CA in the membrane medium increased over time, thus implying that the CA-induced disaggregation of fibrils depends on the mimicked environment.

Conclusions: CA exhibited strong anti-amyloidogenic effect in the presence of in vitro membranes. Therefore, CA revealed to be a promising candidate to prevent and cure AD patients. Further in vivo studies are planned to validate the therapeutic potential of this nutrient.
ON-DEMAND SYMPOSIUM: AD DIAGNOSIS, IMAGING & TRANSLATIONAL DRUG DEVELOPMENT

IMMUNOPET IMAGING OF AMYLOID-BETA IN THE TGF344-AD RAT MODEL

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Aims: Hijacking the transferrin receptor (TfR) to transport antibody-based amyloid-beta (AB) positron emission tomography (PET) radioligands across the blood-brain barrier (BBB) has only been demonstrated in Alzheimer disease (AD) mouse models. The main aim of this study was to test if this strategy is possible in an AD rat model. The affinity to TfR is described to influence BBB transport of therapeutic ligands. Thus, the second aim was to assess the role TfR affinity plays in BBB transport of antibody-based PET radioligands.

Methods: F(ab')₂ fragments of the AB antibody, Bapineuzumab (Bapi), were chemically conjugated to one of two affinity variants for the rat TfR antibody, OX26 (OX26₅ or OX26₇₆), to create two bispecific fusion proteins: OX26₅-F(ab')₂-Bapi and OX26₇₆-F(ab')₂-Bapi. Brain uptake of the two [¹²⁵I]OX26-F(ab')₂-Bapi variants was determined 4 and 70h post-injection into wild-type F344 (WT) rats. In vivo PET imaging was conducted in TgF344-AD and WT rats with [¹²⁴I]OX26-F(ab')₂-Bapi.

Results: Pharmacokinetic studies indicated that more [¹²⁵I]OX26₅-F(ab')₂-Bapi was taken up into the brain 4h post-administration than [¹²⁴I]OX26₇₆-F(ab')₂-Bapi (Figure 1A). [¹²⁴I]OX26₅-F(ab')₂-Bapi visualized AB pathology with PET in the TgF344-AD rat model. Furthermore, the PET signal in the TgF344-AD rats was significantly higher in all brain regions measured compared to WT littermates with no AB pathology.
Conclusions: Antibody-based PET imaging of brain AB pathology using TfR-mediated transport was successful in an AD rat model suggesting that this strategy could be translated from bench to bedside with the correct human anti-TfR antibody.
ON-DEMAND SYMPOSIUM: AD DIAGNOSIS, IMAGING & TRANSLATIONAL DRUG DEVELOPMENT

APP MRNA G-QUADRUPLEX STABILIZERS: A PATH TO TREAT AMYLOID PATHOLOGIES

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Aims: Amyloid precursor protein (APP) regulates neuronal synapses and its cleavage product Aβ is linked to Alzheimer’s disease. The intense investigation by the scientific community has centered on understanding the molecular pathways that underline the production and accumulation of Aβ. Therapeutics that reduce the level of this plaque-promoting peptide may reduce the ongoing neural dysfunction and degeneration that occurs so profoundly in AD. Several studies also support the notion that overproduction of APP underlies AD. Elevated APP mRNA levels can result from altered APP transcription, although the specific transcription factors involved remain elusive. By contrast, there is extensive evidence that APP expression is potently regulated by post-transcriptional mechanisms such as APP mRNA stabilization and APP translation, indicating that the regulation of APP mRNA metabolism is an important event in AD pathophysiology. Based on this evidence we aim to abrogate APP overexpression in prodromal phase of pathology making this event a key player for the pathology.

Methods: FMRP displays the presence of specific mRNA recognition sites characterized by an RGG box that can bind a guanine-rich RNA. The presence of a clear picture of the peptide/mRNA interactions raises the opportunity to design specific peptides that can bind and stabilize the target mRNA in its specific duplex/G4fold which will be probed in silico and tested in vitro.

Results: Preliminary results demonstrated the reduction of APP expression upon 6h of drug exposition in neuronal primary culture.

Conclusions: We screen the most efficient able to reduce the APP expression aiming to use as potential therapeutic strategy for this pathology affecting most of people worldwide.
DIFFERENCES IN MACULAR VESSEL DENSITY ASSESSED BY OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY ACROSS COGNITIVE IMPAIRMENT: DATA FROM THE NORFACE COHORT

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Aims: Optical Coherence Tomography angiography (OCT-A) allows the detection of retinal vessel density (VD) loss, which is a reflection of brain vascular pathology. We aimed to investigate differences of macular VD in the superficial plexus in a cohort of individuals cognitively unimpaired (CU), with amnestic probable Mild Cognitive Impairment (MCI), probable Alzheimer’s dementia (AD) and Vascular Dementia (VaD).

Methods: Clinical, demographical, ophthalmological and OCT-A data from the Neuro-ophthalmology Research at Fundació ACE (NORFACE) project in Barcelona, Spain were analyzed. Differences of macular VD from the superficial plexus in four quadrants (superior, nasal, inferior and temporal) among diagnostic groups (CU, MCI, AD and VaD) were assessed in a multivariate regression model, adjusted by age, sex, education, hypertension, diabetes mellitus and stroke. The interaction of sex and diagnosis in predicting VD differences, as well as the correlation of VD with MMSE scores were also investigated.

Results: The cohort comprised 561 participants: 128 CU, 120 MCI, 257 AD and 56 VaD. Regression models showed only a significantly higher VD in the temporal quadrant in MCI compared to CU participants (49.10±4.91 vs 47.22±4.17, p=0.02, d=0.31). Age, sex, education, hypertension, diabetes mellitus and stroke had no significant contribution to VD variability. The interaction of sex and diagnosis had no effect in differentiating VD. MMSE scores were not correlated to VD (all r<0.15; p>0.11).

Conclusions: Our study does not support the usefulness of macular VD, assessed by OCT-A, as a biomarker of vascular pathology discriminating cognitive groups, showing only moderate VD differences between CU and MCI individuals in the temporal quadrant.
ON-DEMAND SYMPOSIUM: DISEASE MECHANISMS, INFLAMMATION. MICROGLIA, APOE ASTROCYTES, AMYLOIDS, LIPIDS, FATTY ACIDS 2

HUMAN-DERIVED MICROGLIA TO STUDY GENETICS, DEVELOPMENT AND DISEASE

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Aims: Microglia are critically involved in complex neurological disorders with a strong genetic component, such as Alzheimer’s disease, Parkinson’s disease and Frontotemporal dementia. While mouse microglia can recapitulate aspects of human microglia physiology, they do not fully capture the human genetic aspects of disease nor reproduce all human cell states. However, primary cultures of human microglia or microglia derived from human pluripotent stem cells (PSC) are difficult to maintain in brain-relevant cell states in vitro. To address these hurdles we recently described MIGRATE (Microglia In vitro Generation Refined for Advanced Transplantation Experiments), a combined in vitro differentiation and in vivo xenotransplantation protocol to study human microglia in the context of the mouse brain. We demonstrated that the transplanted cells recapitulate transcriptionally human primary microglia ex vivo, are capable to react to challenges and show expression of human-specific Alzheimer’s disease risk genes. Although MIGRATE yields up to 80% chimerism consistently across different PSC lines, optimal engraftment efficiency exclusively results from the first harvest time-point in vitro.

Methods: To further characterize the harvest capable to colonize the in vivo brain environment, we used bulk and single-cell transcriptomics approaches to explore the heterogeneity of PSC-derived microglial progenitors populations in vitro.

Results: Here, we report fundamental differences in sequential harvests that recapitulate key aspects of human microglia development.

Conclusions: We anticipate that MIGRATE coupled to genetic manipulations and multi-omics approaches could address a variety of questions concerning normal physiology, development and pathology of human microglia in the in vivo environment of the brain.
SPATIOTEMPORAL DISTRIBUTION OF BACE1 AND ITS ASSOCIATION WITH GLIAL, NEURAL AND MICROVASCULAR PATHOLOGIES IN RAT BRAIN FOLLOWING TBI

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Aims: Traumatic brain injury (TBI) is a significant risk factor for development of Alzheimer's Disease and Alzheimer's Disease Related Dementias (AD/ADRD). β-site amyloid precursor protein cleaving enzyme 1 (BACE1), also known as β-secretase, in etiopathology of AD/ADRD. However, the role of BACE1 in TBI is poorly understood. Thus, the goal of this study was to further investigate pathological mechanisms that can contribute to development of AD/ADRD following TBI focusing on role of BACE1 as a potential therapeutic target to prevent secondary injury.

Methods: Quantitative immunohistochemistry (IHC) was performed in paraffin embedded serial brain slices obtained upto 3 months after controlled cortical impact (CCI) in adult Sprague Dawley rats. Analyses focused on spatiotemporal profiles of BACE1 and its association with different IHC markers.

Results: The results demonstrated that CCI caused upregulation of BACE1 and cleaved-caspase-3 with differential spatiotemporal profiles in ipsilateral cortex, hippocampus, thalamus and corpus callosum. Interestingly, the BACE1 upregulation was observed in areas of accumulation of acute microhemorrhages and chronic microbleeds determined by histological stains. Acute upregulation of cleaved-caspase-3 was observed primarily intracellularly, whereas at chronic time points cleaved-caspase-3 immunoreactivity was detected as extensive puncta and extracellular aggregates with maximal accumulation in selected brain regions. BACE1 and cleaved-caspase-3 upregulation showed different spatiotemporal associations with glial, neural and microvascular markers.

Conclusions: This study provides first demonstration of spatiotemporal upregulation of BACE1 and evolving upregulation of cleaved-caspase-3 and BACE1 representing independent, though overlapping, pathways associated with activation of secondary brain injury and neurodegeneration as possible mechanisms contributing to an increased risk of AD/ADRD following TBI.
TARGETING ASTROCYTES ALLEVIATES CEREBROVASCULAR DYSFUNCTION IN A MOUSE MODEL OF SMALL CEREBROVESSEL DISEASE

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Aims: We've shown previously that inhibition of NFAT transcription factors in reactive astrocytes preserves synaptic function in mouse models of Alzheimer's disease (AD) and related dementias (ADRD). Here, we used two photon imaging and other in vivo approaches to assess the impact of astrocytic NFAT signaling on cerebrovascular dysfunction in a hyperhomocysteinemia (HHcy) diet model of small cerebrovessel disease.

Methods: C57BL/6J mice were fed with control diet (CT) or diet that was deficient in B6 and B9 vitamins (and enriched in methionine) to induce HHcy and cerebrovascular pathology. Some mice received intracortical or intrahippocampal injections of AAV-Gfa2 to drive expression of VIVIT (an NFAT inhibitor) or EGFP (control) specifically in astrocytes. Other mice did not receive AAV-Gfa2-VIVIT or EGFP, but instead were injected three weeks before endpoint with AAV-Gfa104-GCaMP6f to visualize Ca\textsuperscript{2+} fluctuations in astrocytes. 2 photon imaging was used to assess astrocyte reactivity, astrocyte Ca\textsuperscript{2+} fluctuations, and neurvascular coupling (NVC) in barrel cortex (Ca\textsuperscript{2+} and NVC were assessed in fully awake mice). In another cohort of CT and HHcy diet mice, the effect of AAV-Gfa2-VIVIT or EGFP was assessed on open field behavior and Y maze performance.

Results: Treatment with HHcy diet led to progressive astrocyte reactivity and an increased number of spontaneous astrocytic Ca\textsuperscript{2+} transients in the barrel cortex of awake mice. HHcy diet also caused deficits in NVC (elicited by whisker stimulation) and Y maze performance, which were attenuated by inhibition of astrocytic NFATs.

Conclusions: The results suggest that reactive astrocyte signaling contributes to cerebrovascular dysfunction in ADRD.
ON-DEMAND SYMPOSIUM: DISEASE MECHANISMS, INFLAMMATION. MICROGLIA, APOE ASTROCYTES, AMYLOIDS, LIPIDS, FATTY ACIDS 2

NOVEL NLRP3 INHIBITORS AS POTENTIAL THERAPEUTICS FOR ALZHEIMER'S DISEASE

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Aims: NLRP3 inflammasome is a cytosolic protein complex that plays essential roles in innate immune responses. Dysregulation of this protein complex has been linked to development of neuroinflammation in Alzheimer's disease (AD). Therefore, NLRP3 inhibitors represent promising AD treatments. Two aims are proposed to this study: Aim 1 is to elucidate the mechanisms of action for our current lead NLRP3 inhibitors. Aim 2 is to structurally optimize the current lead compounds.

Methods: For aim 1, photoaffinity labeling probes of the lead NLRP3 inhibitor will be designed and synthesized. Then, photoaffinity labeling and mass spectrometry analysis will be conducted. Cellular colocalization and thermal shift assay will also be performed. For aim 2, small molecule compounds based on the lead structure will be designed, synthesized, and biologically characterized using biochemical, biophysical, and cellular assays.

Results: Mechanistic studies demonstrated that our NLRP3 inhibitors target the LRR domain of NLRP3, representing a novel and first-in-class mechanism of action. Furthermore, the results demonstrated that chemical scaffold can be managed to provide both covalent and non-covalent inhibitors. A series of analogs were successfully synthesized and characterized. The biological characterization led to identification of new lead inhibitors with significantly improved potency and binding affinity.

Conclusions: In conclusion, a novel scaffold was identified with unique binding interactions to the LRR domain of NLRP3, and the flexibility to provide both covalent and noncovalent NLRP3 inhibitors. Medicinal chemistry studies confirmed that the scaffold can be optimized to improve inhibitory potency and binding affinity to the NLRP3 protein, and new lead NLRP3 inhibitors have been identified for further development.
ON-DEMAND SYMPOSIUM: DISEASE MECHANISMS, INFLAMMATION. MICROGLIA, APOE ASTROCYTES, AMYLOIDS, LIPIDS, FATTY ACIDS 2

TARGETING NEUROINFLAMMATION: PRECLINICAL PHARMACOKINETICS AND PHARMACODYNAMICS OF A REFORMULATED ANTI-INFLAMMATORY COMPOUND

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Aims: Much research has focused on neuroinflammation in the pathogenesis of various CNS disorders. Many attempts have been made to repurpose non-steroidal anti-inflammatory drugs (NSAIDs), particularly for Alzheimer’s disease, with limited success. As NSAIDs have been formulated for minimal blood-brain-barrier penetration, there is potential to reformulate anti-inflammatory agents to improve delivery and uptake in the CNS. Here we present findings from preclinical pharmacokinetic (PK) and pharmacodynamic (PD) studies that support development of a lipid-based anti-inflammatory reformulation (MT1980).

Methods: 1) PK properties of MT1980 vs standard (non-reformulated drug), were investigated in C57BL/6 mice. Blood samples were taken to determine mean blood concentrations, Cmax, Tmax, AUC and t1/2. Terminal brain samples were taken 2 hours post-dose to measure brain levels of MT1980 versus standard. 2) Efficacy of MT1980 to reduce neuroinflammation was investigated following 4-day exposure to endotoxin lipopolysaccharide in C57BL/6 mice. Inflammatory cytokines included IL-1β, IL-6, IL-10, TNF-α, MCP-1 and MCP-2.

Results: PK – absorption profiles in blood demonstrated significant improvement over standard drug. At the two highest dose levels of MT1980, significantly higher levels of drug were measurable in brain, compared to standard. PD – in plasma both MT1980 and standard drug significantly inhibited cytokine levels. However, in brain tissue, only MT1980 had a significant inhibitory effect for reducing cytokine concentrations.

Conclusions: Across two preclinical studies, MT1980 demonstrates significant PK and PD improvements compared to standard drug. The increased ability of MT1980 to cross the blood-brain-barrier in-vivo and furthermore, significantly decrease expression of inflammatory cytokines suggests it has potential utility for treating neuroinflammatory-related indications.
ON-DEMAND SYMPOSIUM: DISEASE MECHANISMS, INFLAMMATION. MICROGLIA, APOE ASTROCYTES, AMYLOIDS, LIPIDS, FATTY ACIDS 2

ADENOSINE A2A RECEPTOR ANTAGONISTS NEGATIVELY REGULATES NMDA RECEPTOR IN ALZHEIMER DISEASE

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Aims: N-methyl D-aspartate ionotropic glutamate receptor (NMDAR), which is one of the main targets to combat Alzheimer’s disease, is expressed in both neurons and glial cells. The aim of this paper was to assess whether the adenosine A2A receptor (A2AR), which is a target in neurodegeneration, may affect NMDAR functionality.

Methods: Biophysical, biochemical, signaling and immuno-cyto/histochemical assays were performed in a heterologous cell expression system and in primary cultures of neurons and microglia (resting and activated) from control and the APPSw,Ind transgenic mice.

Results: On the one hand, the receptors are able to physically interact forming receptor complexes, mainly in microglia. In fact, the amount of complexes is markedly enhanced in activated microglia. On the other hand, the interaction results in a novel functional entity that displays a cross-antagonism, that could be useful to prevent exacerbation of NMDAR function by using A2AR antagonists. Interestingly, the amount of complexes was markedly higher in the hippocampal cells from APPSw,Ind than from control mice. In neurons, the number of complexes is lesser, probably due to NMDAR not interacting with the A2AR. However, activation of the A2AR receptors resulted in higher NMDAR functionality in neurons, probably by indirect mechanisms.

Conclusions: The results suggest that A2AR antagonists, that are already approved in Parkinson’s disease, have potential to afford neuroprotection in AD in a synergistic-like fashion. i.e. via both neurons and microglia.
Aims: The neuropathology of Alzheimer’s disease (AD) is characterized by hyperphosphorylated tau neurofibrillary tangles and amyloid beta (Aβ) plaques. Aβ plaques are hypothesized to follow a development sequence starting with diffuse plaques, which evolve into more compact plaques and finally mature into the classic cored plaque type. A better molecular understanding of Aβ pathology is crucial, as the role of Aβ plaques in AD pathogenesis is under debate.

Methods: We studied the deposition and fibrillation of Aβ in different plaque types with label-free infrared imaging. Fourier-transform infrared (FTIR) imaging was performed on native snap-frozen brain tissue sections from AD cases and non-demented control cases. Subsequently, the scanned tissue was stained against Aβ and annotated for the different plaque types by an AD neuropathology expert. In total, 160 plaques (68 diffuse, 32 compact, and 60 classic cored plaques) were imaged with FTIR.

Results: In diffuse plaques, we detect evidence of short antiparallel β-sheets, suggesting the presence of Aβ oligomers. Aβ fibrillation significantly increases alongside the proposed plaque development sequence. In classic cored plaques, we spatially resolve cores containing predominantly large parallel β-sheets, indicating Aβ fibrils.

Conclusions: Combining label-free infrared imaging and immunohistochemistry on brain tissue samples of AD and non-demented cases provides novel insight into the spatial distribution of the Aβ conformations in different plaque types. This way, we reconstruct the development process of Aβ plaques in human brain tissue, provide insight into Aβ fibrillation in the brain, and provide new implications for therapeutic approaches.
THE EFFECTS OF ANTIGEN PRESENTATION ON SYSTEMIC AND LOCAL IMMUNE RESPONSES IN AN ALPHA-SYNucleIN SEeded MODEL OF PARKINSON’S DISEASE

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Aims: Approximately 95% of all Parkinson’s disease (PD) cases are sporadic and have a multifactorial etiology where genetics, lifestyle and environment affect the risk of developing PD. To date there are no treatments available targeting the progression of PD. PD hallmarks include degeneration of dopaminergic neurons in the substantia nigra (SN), α-synuclein (α-syn) pathology and neuroinflammation. We aim to elucidate how genetic variation regulating MHCII expression affects local and systemic immune populations in an α-syn seeded model of PD in rats.

Methods: DA.VRA4 congenic rats with single nucleotide polymorphisms in the Mhc2ta gene encoding the master regulator of MHCII expression were compared to DA background strain. To model PD rAAV vector overexpressing α-syn was unilaterally injected into the SN followed by striatal seeding with α-syn pre-formed fibrils (PFFs), two weeks later. ELISA and flow cytometric analysis were used to study local (brain) and peripheral (blood) changes in cytokine levels and immune populations at four- and eight-weeks post nigral injection (Fig1).

Results: Flow cytometric analysis of immune populations in brain tissue revealed that prior to initiating the PD model, microglia from DA.VRA4 rats expressed lower MHCII levels compared to DA. However, the number of MHCII+ microglia were higher in DA.VRA4 rats compared to DA. Microglia, peripheral immune populations and cytokine levels are currently being analyzed after α-syn transgene- and PFF administration. Immune populations identified include T-lymphocytes and cells of myeloid lineage.

Conclusions: Mhc2ta affects both number of microglia and MHCII levels under normal conditions and could affect the neuroinflammatory response to PD-like pathology.
Aims: The number of people with neurological disorders continues to rise. Dementia patients alone number about 50 million globally, with 8 million new cases annually. The cost of managing CNS disorders in the US and EU is upwards of $2 trillion. Yet disease-modifying drugs are still not available. Objectives: Lipid peroxidation (LPO) is the common denominator of various genetic and idiopathic neurological diseases, including AD, PD, HD, FA, ALS, PSP, tauopathies, etc. LPO oxidizes polyunsaturated fatty acids (PUFA) through a chain reaction autoxidation process, generating multiple damage. PUFAs form cellular, mitochondrial, lysosomal and numerous other membranes which are compromised by LPO. The toxic products formed through LPO, such as reactive carbonyls, can stay in membranes or diffuse into aqueous cross-linking proteins and wreaking further damage. Antioxidants cannot stop LPO for stoichiometric reasons. A novel approach to keeping the LPO in CNS disorders is needed.

Methods: Regioselective deuteration that reinforces oxidation-prone sites of PUFAs is a novel, non-antioxidant treatment modality that reduces LPO.

Results: Rodent models demonstrate the efficiency of D-PUFAs in preserving the intact membranes and mitigating various pathologies, including AD, PD, Huntington’s disease and retinal conditions.

Conclusions: D-PUFAs are non-toxic and show potential as a new class of drugs for neurological treatment. Animal data and ongoing human trials in PSP, ALS, FA and INAD will be discussed.
ON-DEMAND SYMPOSIUM: PD, MSA ANIMAL MODELS, MECHANISTIC ASPECTS AND THERAPEUTIC STRATEGIES

REPURPOSING THE ANXIOLYTIC DRUG BUSPIRONE TO COUNTERACT INFLAMMATION IN CELLULAR AND ANIMAL MODELS OF PARKINSON'S DISEASE

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Aims: Considerable evidence suggests that blockade of the dopamine-3-receptor (D3R) is neuroprotective and reduces inflammation in models of Parkinson's disease (PD). However, to date there are no selective D3R antagonists in the market. Using CRISPR-Cas9 technology we generated stable Drd3⁻/⁻ and Htr1a⁻/⁻ BV-2 microglial cell lines, we showed that buspirone counteracted LPS-induced NO release (p<0.001), IL-1β (p<0.01) and TNF-α (p<0.0001) gene expression in WT cells, whereas it exerted limited effects in Drd3⁻/⁻ and Htr1a⁻/⁻ microglia.

Methods: To determine if buspirone elicited neuroprotective effects in vivo, C57BL/6 mice were treated with the PD-mimetic rotenone (10mg/kg rotenone i.p. × 21 days) also received daily injections of either 1, 3, or 10mg/kg buspirone for 21 days.

Results: Buspirone treatment successfully mitigated rotenone-induced deficits in locomotor and exploratory behaviours in the Open Field test. Additionally, we found that rotenone caused variable degrees of toxicity across the different brain regions examined (i.e. midbrain, striatum, prefrontal cortex, amygdala, hippocampus and spinal cord) and these effects were ameliorated by buspirone co-treatment. In the midbrain, buspirone successfully restored inflammation and oxidative stress to levels comparable to healthy mice, as shown by a decrease in CD11b (p<0.001), IL-1β (p<0.0001), SOD1 (p<0.001) and GFAP (p<0.01). The drug also prevented dopaminergic cell loss in the midbrain (TH expression, p<0.0001) and altered the expression of endogenous neurotrophic molecules such as the neuropeptides PACAP and VIP and the neurotrophic factors BDNF and ADNP.

Conclusions: In summary, our findings indicate that buspirone attenuates microglial polarization after LPS challenge and can mitigate rotenone-induced neurotoxicity and inflammation in vivo.
Aims: Alpha-synuclein (aSyn) aggregation at synapses is considered a key event contributing to nigrostriatal neurons degeneration in Parkinson’s disease (PD). We found that Synapsin III (syn III), a synaptic phosphoprotein regulating dopamine (DA) release together with aSyn, composes aSyn fibrils in the brain of PD patients and participates in aSyn aggregation in experimental models of PD. We observed that syn III reduction results in aSyn aggregates decrease and rescues PD-like striatal and motility deficits in a human aSyn transgenic (tg) mouse model. Finally, we found that the motility redemption observed in tg mice following the administration of methylphenidate (MPH) is independent on DA transporter inhibition but is lost upon syn III silencing. These findings support that syn III could constitute a druggable therapeutic target for PD.

Methods: We synthesized compounds displaying elevated “in silico” syn III-binding ability and assessed if they could stimulate functional syn III/aSyn interaction and reduce the pathological syn III/aSyn interplay, thus resulting in the decrease of aSyn aggregates in in vitro models of PD. The most efficient compounds were tested in a human aSyn tg model to assess their ability to reduce aSyn aggregation and rescue the PD-like phenotype.

Results: Two compounds were able to stimulate the functional aSyn/syn III interaction without exerting toxic effects. Notably, these compounds were able to inhibit aSyn aggregation in in vitro and in vivo models of synucleinopathy.

Conclusions: These findings support that molecules targeting syn III could represent promising therapeutic approaches for counteracting aSyn aggregation, dopaminergic dysfunction and degeneration and behavioural deficits in PD.
ON-DEMAND SYMPOSIUM: PD, MSA ANIMAL MODELS, MECHANISTIC ASPECTS AND THERAPEUTIC STRATEGIES

REDUCTION OF ALPHA-SYNUCLEIN PATHOLOGY IN A MOUSE MODEL OF PD USING A BRAIN-PENETRATING BISPECIFIC ANTIBODY

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Aims: Immunotherapy targeting the toxic, aggregated alpha-synuclein (αSYN) is one of the most promising approaches in treatment of synucleinopathies such as Parkinson’s disease (PD). However, brain penetration of antibodies is hampered by their size. Here, we have used RmAbSynO2-scFv8D3, a modified bispecific antibody targeting aggregated αSYN as well as the transferrin receptor for facilitation of brain uptake, aimed at improving treatment efficacy.

Methods: Uptake of the bispecific antibody following intraperitoneal and intravenous injections was compared by measurement of I-125 labelled antibodies in brain at different time points, and in extracellular fluid (ECF) by microdialysis. Female Thy1-αSYN mice (15-16 months old, n=7-8/group) were intravenously injected with either 10 mg/kg of RmAbSynO2-scFv8D3, 10 mg/kg unmodified RmAbSynO2 or PBS in a short-term dosing regimen with three injections during five days. Brains were analyzed biochemically with enzyme-linked immunosorbent assay (ELISA).

Results: Intravenous injections of [125I]RmAbSynO2-scFv8D3 resulted in significantly higher standardized uptake value (%injected dose/bodyweight, SUV) compared to intraperitoneal injections, and approximately 40x more than the unmodified variant (Fig 1A). Higher %SUV for intravenously injected [125I]RmAbSynO2-scFv8D3 compared to intraperitoneal injections was also noted in ECF sampled by microdialysis (Fig 1B).

Levels of aggregated αSYN in the brain following treatment with RmAbSynO2-scFv8D3 was significantly decreased in TBS-T extracts of cortex and midbrain compared to PBS as measured by ELISA (Fig 2).
Conclusions: This study demonstrates that the intravenous route is preferred for fast and high brain uptake of engineered antibodies. Furthermore, facilitated brain uptake of αSYN antibodies can improve treatment efficacy, requiring lower doses and thereby reducing systemic side effects.
ON-DEMAND SYMPOSIUM: PD, MSA ANIMAL MODELS, MECHANISTIC ASPECTS AND THERAPEUTIC STRATEGIES

ALTERED AMPA RECEPTOR TRAFFICKING IN STRIATAL NEURONS ASSOCIATED WITH PARKINSON'S DISEASE LRRK2-G2019S MUTATION

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Aims: Imaging in human PD patients shows altered corticostriatal connectivity. In mice, a knockin mutation of LRRK2\textsuperscript{G2019S} disrupts long-term potentiation (LTP) of cortical synapses onto striatal projection neurons (SPNs), a deficit that may contribute mechanistically to cognitive and psychiatric non-motor symptoms of PD in humans. LRRK2\textsuperscript{G2019S} has been implicated in intracellular trafficking defects. We have therefore examined how LRRK2\textsuperscript{G2019S} impacts AMPAR subunit trafficking at identified SPN synapses.

Methods: Using a combination of approaches applied to corticostriatal co-cultures or acute corticostriatal slices, we tracked and quantified GluA subunit distribution and function in WT and Lrrk2\textsuperscript{G2019S} SPNs at baseline and in response to LTP.

Results: At baseline, mutant SPNs exhibit a higher ratio of surface GluA1 to GluA2 subunits and, consistent with this, D1R SPNs show a greater sensitivity to NASPM (an antagonist of calcium-permeable AMPAR subunits) compared to WT SPNs. Mutant D2R-SPNs were similar to WT. Following chemical-LTP stimulation in culture, GluA1 was trafficked to the surface in WT SPNs as expected, but failed to do so in mutant SPNs. Ongoing experiments will identify the arm of intracellular trafficking (endocytosis vs. recycling) that may be defective.

Conclusions: LRRK2\textsuperscript{G2019S} dysregulates the delivery and maintenance of postsynaptic GluA subunits at baseline and under activity-dependent conditions of long-term plasticity, a deficit that could contribute to cognitive deficits and psychiatric symptoms in PD.
"NEUROPROTECTION BY PHARMACOLOGICAL STABILIZATION OF PINK1"

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Aims: PTEN-induced kinase 1 (PINK1) is mutated in some patients with early-onset familial Parkinson's disease (PD). PINK1 plays a unique role in supporting dendritic structures and its overexpression is neuroprotective. Increasing endogenous PINK1 levels and/or activity would represent valuable strategies for assessing PINK1 function and developing future therapies. The novel small molecule BC1464 blocks association between E3 ubiquitin ligase FBXO7 and PINK1, leading to increased cellular PINK1 concentrations. We hypothesize that BC1464 will protect cells against a variety of neurodegenerative disease stressors.

Methods: Human neuroblastoma cell line SH-SY5Y, transfected primary neurons or cells derived from patients with PD were treated with DMSO vehicle, active compound BC1464, or inactive compound BC1465. Cell viability was determined via alamar blue assays 24 hours after treatment and neurite lengths measured using ImageJ.

Results: Active compound BC1464 elicited increased cell viability and neurite length in SH-SY5Y cells and primary neurons after exposure to MPP+ compared to inactive compound or vehicle. The compound also protected against toxin-induced cell death in PD patient-derived neural progenitor cells and fibroblasts, with promising results observed against dendritic shortening elicited in genetic models of PD and other Alzheimer's-related diseases.

Conclusions: Pharmacological stabilization of PINK1 is protective in culture models of PD. Interestingly, PINK1 is decreased in postmortem tissue from patients with sporadic neurodegenerative diseases. Ongoing work is aimed at testing the protective potential of BC1464 and the underlying mechanism of PINK1-mediated protection in a variety of genetic neurodegeneration models.
ON-DEMAND SYMPOSIUM: PD, MSA ANIMAL MODELS, MECHANISTIC ASPECTS AND THERAPEUTIC STRATEGIES

MINI-STRIATAL CIRCUITS ON CMOS-MEA CHIPS FOR HIGH-THROUGHPUT ELECTROPHYSIOLOGICAL PHENOTYPING OF PARKINSON'S DISEASE

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Aims: Despite the tremendous efforts made by clinical geneticists and epidemiologists, the cause of most Parkinson’s disease (PD) cases remains unknown. We hypothesize that the molecular alterations that cause PD manifest in characteristic electrophysiological phenotypes in disease-relevant neuronal circuits. To test this, we are building synthetic nigrostriatal circuits on top of CMOS multielectrode array (MEA) microchips.

Methods: We generated cortical, striatal, and ventral midbrain neural progenitors from induced pluripotent stem cells, seeded onto specific locations of the CMOS-MEA chips, and primed them to differentiate into their corresponding neuronal and glial progeny. We engineered knock-in fluorescent reporter systems that enabled us to monitor the birth of the different types of neurons and the establishment of physical connections through their axons and dendrites. Using the chip's electrodes, we evaluated how neurons acquire electrophysiological and synaptic maturity. In addition, by applying a combination of receptor agonists and antagonists as well as enabling the electrodes to stimulate specific subsets of neurons, we can unravel the functional connectivity as the network develops.

Results: Region-specific neural progenitors efficiently differentiated into striatal projection, cortical excitatory, and nigral dopamine neurons. Characterization of the network revealed that the different neuronal subtypes established functional connections with their in vivo targets, which led to progressive circuit synchronization over time.

Conclusions: Our brain on a chip approach is reproducible, robust, and recapitulates relevant hallmarks of the striatal circuit. We are now in position to use our mini-striatal circuits to functionally phenotype idiopathic PD.
LONGITUDINAL IN VIVO RECORDINGS OF ELECTROPHYSIOLOGICAL CHANGES IN A MOUSE MODEL OF PROGRESSIVE ALPHA-SYNUCLEINOPATHY

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Aims: We established an in vivo electrophysiological recording paradigm to uncover neurofunctional changes in a mouse model of progressive alpha-synuclein (aSyn) pathology and explore their association with neuropathology.

Methods: Spreading of aSyn pathology was induced by injecting preformed human aSyn fibrils (PFF) into the striatum of young Thy1-h[wt]aSyn (line14) transgenic mice. A recording electrode was implanted into the contralateral striatum and EEG was assessed on the ipsilateral motor and parietal cortex over 0-3 (early cohort) and 3-6 months post injection (late cohort). Brain sections were immunohistochemically processed to analyze aSyn pathology (pSer129), dopaminergic neurodegeneration (TH), and microgliosis (Iba1).

Results: Alpha-synuclein pathology developed in neuroanatomically connected brain regions, particularly in midbrain, hypothalamic and pontine nuclei, which was associated with increased microgliosis. No TH-loss was observed in the substantia nigra or striatum in PFF injected mice when compared to the vehicle group. Overall locomotor activity was unaffected by the progressive aSyn pathology, but the aging of the mice was accompanied by reduced locomotor speed. Longitudinal in vivo recordings revealed progressive functional changes in both groups and age cohorts. We continue to investigate the association of functional changes with neuropathological alterations and aging.

Conclusions: The aSyn pathology spreading model in mice can be combined with longitudinal recordings of electrophysiological changes in several brain regions. If in vivo functional readouts are associated with neuropathology, this proposed translational animal model may allow for monitoring electrophysiological biomarkers during disease progression and reveal the effects of experimental therapeutics on both the functional and histological level.
ON-DEMAND SYMPOSIUM: PD, MSA ANIMAL MODELS, MECHANISTIC ASPECTS AND THERAPEUTIC STRATEGIES

THE THY1-ASYN MOUSE MODEL OF PARKINSON’S DISEASE REPLICA TES THE CLINICAL FAILURE OF VENGLUSTAT AND APPEARS AS A RELEVANT PRECLINICAL MODEL WITH TRANSLATIONAL PREDICTABILITY

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Aims: Based on the association of glucocerebrosidase (GBA) dysfunction with Parkinson’s disease (PD), it was hypothesized that the reduction of the enzyme’s substrate, GlcCer, would act as disease-modifying treatment strategy. Recently, venglustat, an inhibitor of glucosylceramide synthase, was tested in PD patients with GBA mutations (MOVES-PD). The primary endpoint (MDS-UPDRS) deteriorated faster with venglustat than placebo with possible worsening in the cognitive rating scale, despite successful GlcCer reduction in cerebrospinal fluid and plasma. Clearly the preclinical models used to assess venglustat were not predictive and better ones are required to understand the mechanisms behind venglustat’s clinical failure, a mandatory condition to advance other therapeutical approaches targeting lipid pathways.

Methods: We present an exploratory study of venglustat in an in-depth characterized mouse model of PD, which overexpresses human wild-type alpha-synuclein (Thy1-aSyn mice, Masliah-line-61) and recapitulates alpha-synuclein pathology, dopamine loss and a slow progressive prodromal phase with motor and non-motor symptoms. Thy1-aSyn males and wild-type littermates (n=18/group) were treated from 1.5 to 6 months of age with venglustat as food-admix (60 mg/kg/day), or regular chow. Locomotor abilities and cognition were tested longitudinally followed by comprehensive ex vivo analyses for target engagement, alpha-synuclein pathology and microgliosis.

Results: Despite reducing soluble and membrane bound alpha-synuclein in the striatum, venglustat treatment was unable to revert the anxiogenic and cognitively impaired phenotype of Thy1-aSyn mice and even surprisingly further worsened their motor function.

Conclusions: Our study positions the Thy1-aSyn mice as a relevant model with translational value to support the development of drugs targeting lysosomal pathways.
BEHAVIORAL CHARACTERIZATION OF THE HA53TTG OF PARKINSON’S DISEASE

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Aims: Aggregation of α-synuclein (α-syn) plays a crucial role in Parkinson’s disease (PD) and other synucleinopathies. Point mutations in α-syn have been identified in rare forms of familial PD and are reported to accelerate α-syn oligomerization and aggregation as well as age of symptom onset. Here, we behaviorally characterized human α-syn transgenic mice with A53T mutation (hA53Ttg) developed by Sudhof and colleagues for their activity, motor deficits, emotional learning, and compound muscle action potential (CMAP).

Methods: hA53Ttg mice at an age of 2–6 months were tested for motor deficits in the wire hanging, beam walk and pasta gnawing test, as well as for learning and memory deficits in the contextual fear conditioning test. Additionally, electromyography (EMG) was performed to measure CMAP. Furthermore, several tissues of hA53Ttg animals were and will be analyzed for disease-typical pathological changes.

Results: Already at the age of 2 months, hA53Ttg mice present with severe motor deficits in the wire hanging, Rotarod and beam walk test, while deficits in the pasta gnawing test were significantly altered at the age of 4 months. Changes in emotional learning were prominent in younger mice. Neurofilament-light chain (NFL) levels seem to progressively increase. Further analyses are currently performed.

Conclusions: Our analyses revealed an early motor and learning phenotype in hA53Ttg mice suggesting that this model is ideal for drug testing and early interventions of synucleinopathies. The increased NFL levels suggest a strong neurodegeneration in these mice that needs to be tested in more detail.
ON-DEMAND SYMPOSIUM: PD, MSA ANIMAL MODELS, MECHANISTIC ASPECTS AND THERAPEUTIC STRATEGIES

RCC1-LIKE ABLATION IN DOPAMINERGIC NEURONS YIELDS PROGRESSIVE DEGENERATION AND A PARKINSON’S DISEASE-LIKE PHENOTYPE IN MICE

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Aims: We previously identified RCC1-like as an inner mitochondrial membrane protein important to mitochondrial fusion. Homozygosity for a spontaneous missense Rcc1L mutation causes early embryonic lethality. To test for in vivo importance to neuronal function and whether RCC1L might be implicated in PD pathology, via affecting mitochondrial function, we selectively ablated Rcc1-l in mice in dopaminergic (DA) neurons and tested for PD-like phenotypes, including progressive movement abnormalities, and nigrostriatal track degeneration.

Methods: We created mice lacking the Rcc1-l gene in DA neurons by crossing Rcc1lRcc1l⁰⁰ animals with Slc6a3-Cre mice, in which Cre is expressed from the dopamine transporter gene. Experimental and control groups were examined at 2, 3-4, and 5-6 month postnatally. Animals were tested via open field task to quantify exploratory drive, locomotion, and immobility. Following behavioral testing, animals underwent post-mortem analyses. These included tyrosine hydroxylase (TH) and NeuN immunohistochemistry, Nissl staining, and densitometry analysis of TH expression.

Results: Beginning at 3-months, both female and male Rcc1l⁰⁰/Cre+ mice show rigid muscles and resting tremor, kyphosis and a growth deficit compared with heterozygous, or wild type littermates. Rcc1l⁰⁰/Cre+ mice also have motor impairments at 3 months, progressing until 5-6 months of age. Rcc1l⁰⁰/Cre+ animals show significant age-dependent reduction in locomotion and rearing not seen in either of the controls. These motor impairments are coincident with progressive and significantly reduced TH immunoreactivity in the substantia nigra pars compacta, and loss of striatal DA projections.

Conclusions: We are currently examining mitochondrial fragmentation and depletion in the soma and processes of DA neurons in Rcc1l⁰⁰/Cre+ mice.
Aims: There is evidence that tau adopts distinct aggregate conformations in individual tauopathies and that the biochemical properties of these aggregate structures may drive disease pathogenesis. Unfortunately, we have yet to define the molecular determinants that favor tau assembly into one aggregate conformation over another or predict what subset of those structures are pathogenic. Our aim is to generate a diverse set of tau aggregate structures to define the relationships between tau primary sequence, aggregation properties (e.g. aggregation kinetics, LLPS), aggregate structures, and propagation of those structures.

Methods: We discovered single amino acid substitutions in the wild-type tau sequence drives the formation of distinct tau aggregate structures in vitro. Our goal is now to create thousands of tau variants for in-depth characterization. We developed a high-throughput experimental platform for expression and purification of recombinant tau variants. We have developed small-scale downstream assays to assess aggregation kinetics, aggregate structures and LLPS propensity.

Results: We performed an initial analysis by creating recombinant tau mimics of 37 disease-associated tau missense mutations and found that a subset formed aggregate structures distinct from wild-type tau. Structure was strongly correlated with mutation location within the coding sequence. Some structures are readily propagated over other structures which may have implications for understanding tau strains.

Conclusions: We have built a platform that allows us to systematically dissect relationships between aggregate structure and other aggregate properties which may predict pathogenic potential in vivo. Our long-term goal is to understand how to strategically manipulate the tau sequence to recreate aggregate structures found in tauopathies.
Aims: Tauopathies, including Alzheimer's disease (AD), are a group of neurodegenerative disorders involving Tau hyperphosphorylation. Post-translational modifications of Tau (phosphorylation, truncation...) are essential in their pathogenesis. In this work, we demonstrate the existence of a new, human-specific truncated form of Tau generated by intron 12 retention.

Methods: We detected this isoform using qPCR in brain samples and confirmed the results in RNA-seq samples. We evaluated protein levels by Western blotting in Alzheimer’s patients’ (n=31) and non-demented (n=9) brains. We checked this isoform’s properties, through sarkosyl solubility and microtubule binding assays and its phosphorylation in cultured cells. We explored its modulation through GSK3 and the splicing factor SC35.

Results: We confirmed the detection of a truncated Tau isoform generated by intron 12 retention in human RNA brain samples and in silico studies (383 RNA-seq samples), named W-Tau since it contains a unique 18 amino-acid sequence that includes two tryptophan residues. Analysis of the properties of this isoform shows that it is less prone to aggregation, but is able to bind to microtubules and suffers phosphorylation. Importantly, diminished protein levels of this isoform are found in Alzheimer’s patients’ brains with respect to non-demented individuals. Exploratory findings point towards GSK3 and SC35 being involved in this isoform's expression.

Conclusions: The discovery of a new splicing-generated, non-aggregative, human-specific Tau isoform opens new research avenues to be explored. Its decrease in AD suggests that the lack of this isoform plays an important role in the pathology. Thus, the study of this isoform may help develop future therapies for AD and other tauopathies.
Aims: Alzheimer’s disease (AD) is characterized by the aberrant accumulation of β-amyloid plaques and neurofibrillary tangles of hyperphosphorylated tau (NFTs). Surprisingly, we previously found that the histone demethylase LSD1/KDM1A is mislocalized to NFTs in AD cases. In addition, we showed that loss of LSD1 systemically in adult mice is sufficient to recapitulate many aspects of AD, including widespread neuronal cell death in the hippocampus and cortex, learning and memory defects, and global gene expression changes that match AD cases. Based on these data, we sought to determine whether pathological tau functions through LSD1 to induce neuronal dysfunction.

Methods: If pathological tau is functioning through the sequestration of LSD1, then reducing LSD1 in PS19 Tauopathy mice should make these mice more sensitive to pathological tau, and overexpressing LSD1 should rescue neuronal dysfunction.

Results: Reducing LSD1 in PS19 Tau mice accelerates the depletion of LSD1 from the nucleus. This results in decreased survival, exacerbated paralysis, and increased neurodegeneration. Conversely, overexpressing LSD1 in hippocampal neurons of PS19 mice at 8.5 months, when pathological tau is already present, is sufficient to suppress tau-induced neurodegeneration and block the tau induced immune response through 11 months. Additionally, we find that pathological tau interacts with LSD1 via LSD1’s N-terminal domain.

Conclusions: We propose that pathological tau leads to neuronal cell death in AD by sequestering LSD1 in the cytoplasm and interfering with the continuous requirement for LSD1 to epigenetically repress transcription associated with alternative cell fates. A model for how pathological tau sequesters LSD1 in the cytoplasm will be presented.
Aims: Tau is primarily a cytosolic protein well-known for binding microtubules and regulating microtubule assembly and stability. A large body of evidence indicates that Tau also interacts with the plasma membrane. This interaction has been suggested to play a role in cell signalling and secretion of Tau into the extracellular milieu. However, due to the limitations of conventional microscopy techniques, quantifying how Tau behaves and organises at the plasma membrane has not been possible.

Methods: Using a super-resolution technique called single-particle tracking photoactivated localisation microscopy (sptPALM), we tracked individual molecules of Tau at nanometre and millisecond resolution and investigated their dynamic behaviour near the plasma membrane.

Results: We found in human embryonic kidney and neuroblastoma cell lines that Tau exhibits heterogeneous mobility patterns characterised by confined and free diffusion near the plasma membrane. Preventing Tau-microtubule interactions by either using nocodazole or pseudophosphorylation of Tau at the microtubule-binding domain increased Tau mobility. However, Tau still exhibited heterogeneous mobility patterns, suggesting that this behaviour is associated with the interactions of Tau with components of the plasma membrane. We further found that Tau formed transient hot spots of ~100 nm in diameter, with an apparent lifetime of several tens of seconds. Within the hot spots, the Tau molecules displayed a confined motion, whereas outside the spots, they diffused freely.

Conclusions: Overall, our study provides nanoscale level insight into transient crowding of Tau near the plasma membrane which could be important for understanding the physiological and pathological roles of Tau.
EVALUATION OF ASTROCYTES MORPHOLOGICAL CHANGES IN TAUOPATHIES

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Aims: Despite tau major expression in neurons in physiological condition, abnormal forms of tau protein are also detected in astrocytes in diverse form of tauopathies. Here, we aimed to investigate the consequences of primary neuronal tau pathology on astrocytes morphology.

Methods: We injected intravenously Thy-Tau22 transgenic mice, expressing a double mutant form of the human tau protein in neurons, with a PHP.eB AAV to express tdTomato fluorescent protein specifically in astrocytes. We used three-dimensional (3D) reconstruction to perform a detailed analysis of astrocytes complex morphology in the hippocampus combining confocal microscopy and Imaris software analyses.

Results: At 9 months of age, tau astrocytes’ arborisation is simplified as compared to WT, whereas it is more ramified at 24 months with more branching points. On going 3D sholl analyses show that morphological changes associated to tauopathy are subregion-dependent, with subicular astrocytes being particularly affected. Principal component analysis indicates that the ramification index and number of branches are essential morphological parameters discriminating WT and tau astrocytes. Interestingly, perivascular astrocytic endfeet diameter significantly changed with age in the subiculum.

Conclusions: We expect to unravel how tau pathology-induced morphological changes in astrocytes could affect their functions and interactions with neurons.
ON-DEMAND SYMPOSIUM: TAU, PROTEIN MISFOLDING; ASTROCYTES, DISEASE MECHANISMS & DETECTION

NUCLEAR TAU MODULATES GENE EXPRESSION LEADING TO GLUTAMATE RELEASE ALTERATIONS DURING AD PROGRESSION

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Aims: Neuronal hyperexcitability linked to the glutamate pathway is a peculiar trait of the early stages of Alzheimer’s disease (AD), however, a progressive reduction in glutamate release follows in advanced stages. These events are concomitant with Tau displacement from microtubules followed by toxic protein aggregation thus affecting its subcellular localization. Here we investigated the role of nuclear Tau on glutamatergic dysfunction during AD progression.

Methods: We exploited molecular and imaging techniques to investigate the nuclear localization of Tau and the effect on gene expression of disease-related genes. Moreover, by bioinformatic techniques we studied the Tau-dependent gene expression modulation in the human PFC during the disease progression.

Results: We observed in neuronal cell lines and primary neurons that early pathological destabilization of Tau causes its increase in the nuclear compartment resulting in higher levels of genes involved in the glutamate release pathway thus leading to neuronal hyperexcitability. Moreover, this condition causes a global gene expression alteration mimicking the LMCI stage. On the contrary, late pathological aggregation induces the formation of Tau aggregates in the nuclear compartment. Indeed, aggregated Tau abolishes Tau-dependent increased expression of the glutamate transporter. Remarkably, in the prefrontal cortex of AD patient brains, the glutamate transporter genes are upregulated at early stages and downregulated at late stages.

Conclusions: Our results indicate a direct role of Tau on gene expression that is altered in pathological conditions. Remarkably altered neuronal excitability and glutamatergic release in the PFC during AD progression is linked to the newly discovered function of nuclear Tau.
ON-DEMAND SYMPOSIUM: TAU, PROTEIN MISFOLDING; ASTROCYTES, DISEASE MECHANISMS & DETECTION

TAU SEGMENTATION USING GEOMX DIGITAL SPATIAL PROFILING TECHNOLOGY IDENTIFIES DIFFERENTIALLY EXPRESSED PROTEINS THROUGHOUT THE NEUROFIBRILLARY TANGLE MATURITY LEVELS IN ALZHEIMER’S DISEASE

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Aims: Neurofibrillary tangles are dynamic entities with a lifespan encompassing three maturity levels: intracellular pretangles, intracellular mature tangles, and extracellular ghost tangles. Proteins are differentially expressed throughout the tangle lifespan; however, past studies may be limited by spatial expression or number of proteins investigated. We sought to assess differences in protein expression across the tangle lifespan using GeoMx™ Digital Spatial Profiler (DSP) by NanoString, a new technology allowing for spatially derived multiplex investigation.

Methods: We used DSP to measure protein expression in hippocampi from Alzheimer’s disease (n=6) and nondemented controls (n=2) using the “Human Neural Cell Profiling Core” and “Alzheimer’s Pathology” module. Regions of interest (ROIs) were classified by the major tangle maturity level recognized by a primary fluorescent conjugated tau antibody. Protein expression was normalized to the geometric mean of the three housekeeping genes. The tau-positive segment was specifically analyzed for comparisons.

Results: As expected, cytoskeletal and neuronal markers generally decreased through the tangle lifespan. Neurodegenerative markers generally increased through tangle maturity levels, except for TDP-43 which decreased. Glial markers were generally increased as tangles matured. Interestingly, APOE remained low in pretangles and mature tangles before dramatically increasing in ghost tangles.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Profile</th>
<th>Protein mature (n=6)</th>
<th>Mature Tangle (n=43)</th>
<th>Ghost Tangle (n=38)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFAP</td>
<td>C1FA1</td>
<td>1.10 (1.16, 1.80)</td>
<td>1.16 (1.10, 1.78)</td>
<td>1.79 (1.15, 2.42)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>collagen</td>
<td>C1HA1</td>
<td>1.10 (1.16, 1.80)</td>
<td>1.16 (1.10, 1.78)</td>
<td>1.79 (1.15, 2.42)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>APOE</td>
<td>C1FA1</td>
<td>1.01 (0.83, 1.25)</td>
<td>1.50 (1.25, 1.92)</td>
<td>1.92 (1.50, 2.40)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TDP-43</td>
<td>C1FA1</td>
<td>1.10 (1.16, 1.80)</td>
<td>1.16 (1.10, 1.78)</td>
<td>1.79 (1.15, 2.42)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data reported as median (interquartile range). Representative box plots included below.
Conclusions: Our findings suggest that proteins are differentially expressed throughout the tangle lifespan and the majority followed a monotonically-directed pattern. Non-monotonically directed protein patterns may reflect the death of the neuron during the ghost tangle maturity level. Future studies will test the hypothesis that the microenvironment of the hippocampus is altered with increasing vulnerability to tangle pathology.
Aims: Pathological tau aggregation is a hallmark of several neurodegenerative disorders including Alzheimer's disease. The study and treatment of these tauopathies is hampered by a paucity of tau-specific aggregation modulators. We seek to meet this challenge by discovering and designing drug-like small molecules capable of binding to tau early in its aggregation pathway and inhibiting or reporting on pathological self-assembly. Additionally, we seek to characterize these compounds’ mechanisms of action using functional and biophysical assays. Novel compounds will serve as valuable experimental reagents for studies of tauopathies, and could also serve as leads for the development of tau-targeting therapeutics and diagnostics.

Methods: Tau is an intrinsically disordered protein, rendering traditional medicinal chemistry techniques ineffective. Therefore, we developed novel computational methods to model tau conformation, identify druggable sites, and discover tau-binding compounds. Analog design and synthesis was used to improve affinity and specificity. Fluorescence and millisecond HDX mass spectrometry were used to determine mechanism of action.

Results: We have developed three families of novel tau-binding compounds, some of which interact with monomeric tau and others with fibrillar aggregates. Potencies range from nanomolar to the low micromolar range. Crucially, several of the compounds are substoichiometric inhibitors of aggregation. Moreover, subtle changes in compound structure had major effects on compound potency, highlighting the specific nature of tau-inhibitor interactions.

Conclusions: Our work demonstrates that tau and similar disordered proteins are tractable targets for rational drug design. The resulting compound represent an unrivalled collection of potent, drug-like, tau-specific aggregation inhibitors.
MECHANISMS UNDERLYING THE SELECTIVE VULNERABILITY TO TAU PATHOLOGY IN ALZHEIMER’S DISEASE: FOCUS ON A NOVEL REGULATOR


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Aims: Uncovering molecular origins of selective neuronal vulnerability in Alzheimer’s disease (AD) is important for understanding this devastating disease. Excitatory (EX) neurons in the entorhinal cortex (EC) are preferentially vulnerable to tau pathology. We have previously identified that grid cells (EX neurons in the layers II/III of EC) are specifically vulnerable to tau pathology. One of the molecular characteristics of grid cells is a strong expression of wolframin (WFS1). This study aims to further investigate the molecular mechanisms underlying the role of WFS1 in tau pathology-associated neurodegeneration.

Methods: The immunostaining and WB assay are used to measure the localization and protein levels of WFS1, p-tau, ER stress- and autophagy-lysosome pathway (ALP)-associated proteins. DuoLink assay is used to measure the interaction between WFS1 and tau. TUNEL assay is used for measuring apoptosis. Enriched signaling pathways in WFS1-high EX neurons are defined by analyzing the public snRNA-Seq datasets. The 10x Visium spatial transcriptomics is used to characterize the transcriptomic profiles of pathological tau-associated gene signatures.

Results: Here we report that whole-body knockout of WFS1 increases tau pathology and neurodegeneration in tau transgenic mice; whereas overexpression of WFS1 reverses those changes. WFS1 interacts with tau protein and controls the susceptibility to tau pathology. Furthermore, ER stress- and ALP-associated genes are enriched in WFS1-high EX neurons and are changed in tau transgenic mice with WFS1 deficiency, while overexpression of WFS1 restores them to the normal level.

Conclusions: These results suggest that WFS1 regulates neuronal vulnerability to tau pathology via chronic ER stress and the downstream ALP.
ON-DEMAND SYMPOSIUM: TDP43- AND C9ORF72-RELATED DISEASES 2

TDP-43 PROMOTES AMYLOID-B OLIGOMERIZATION VIA INTERACTION AND WORSENS PATHOLOGY IN A MODEL OF ALZHEIMER’S DISEASE

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**Aims:** TDP-43 inclusions are found in more than half of the Alzheimer’s disease (AD) patients presenting faster disease progression and greater brain atrophy. Previously, we showed full-length TDP-43 forms spherical oligomers and perturbs amyloid-β (Aβ) fibrillation.

**Methods:** To elucidate the role of TDP-43 in AD, here, we examined the effect of TDP-43 in Aβ aggregation and the attributed toxicity in mouse models as well as the interaction between TDP-43 and Aβ.

**Results:** We found TDP-43 inhibited Aβ fibrillization at initial and oligomeric stages. Aβ fibrillization was delayed specifically in the presence of N-terminal domain containing TDP-43 variants, while C-terminal TDP-43 was not essential for Aβ interaction. TDP-43 significantly enhanced Aβ’s ability to impair long-term potentiation and, upon intrahippocampal injection, caused spatial memory deficit. Following injection to AD transgenic mice, TDP-43 induced inflammation, interacted with Aβ, and exacerbated AD-like pathology. TDP-43 oligomers mostly colocalized with intracellular Aβ in the brain of AD patients. In addition, we also investigated the interacting of TDP-43 and Aβ and found the interacting interface for TDP-43/Aβ. Potential interrupting agents were developed.

**Conclusions:** We conclude that TDP-43 inhibits Aβ fibrillization through its interaction with Aβ and exacerbates AD pathology.
ON-DEMAND SYMPOSIUM: TDP43- AND C9ORF72-RELATED DISEASES 2

BIOMARKERS FOR PATIENT STRATIFICATION AND TARGET ENGAGEMENT IN PATIENTS WITH TDP-43 PATHOLOGY

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Aims: Patient identification and stratification as well as early identification of target engagement can be crucial to the development of effective therapies in ALS/FTD. TDP-43 proteinopathy has been described in up to 90% ALS, 50% FTD, 50% Alzheimer's Disease, and 10% of Parkinson's Disease patients. With recent insights into disease mechanisms, it is important to identify patients with TDP-43 pathology for precision medicine therapeutic approaches.

Methods: ALS and healthy control samples were tested in assays to determine the concentration of STMN2 in human biofluids.

Results: We have developed assays to detect STMN2 protein and RNA in human biofluids. The assays have an acceptable limit of detection and can be used as an exploratory biomarker in support of clinical trials.

Conclusions: STMN2 is known to play an important role in axonal growth and maintenance, and restoration of STMN2 expression in neurons with TDP-43 nuclear depletion is capable of rescuing axonal health. Intriguingly, STMN2 splicing changes seen in patients and human cell models are not conserved in lower species. Given this dichotomy, there is no animal model to assist in human dose prediction. Thus, a clinical dose will be based on efficacy in cell-based assays. With this paradigm it is crucial to be able to evaluate target engagement rapidly in clinical studies. We have developed Stathmin-2 based biomarker assays in human biofluids and characterized these markers in ALS patients and healthy individuals. These assays could be useful to identify patients with TDP-43 proteinopathy, and future targeting of these patients for precision medicine-based therapeutics.
THE GRN p.E393A MUTATION LEADS TO PROGRANULIN HAPLOINSUFFICIENCY

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Aims: Heterozygous loss-of-function (LOF) mutations in the progranulin gene (GRN) cause frontotemporal lobar degeneration (FTLD) by a mechanism of haploinsufficiency. For most missense mutations, the contribution to FTLD is not understood. We investigated the effects of the GRN p.E393A mutation. To our best knowledge, this mutation is not yet reported and is absent from mutation databases.

Methods: Mutation identification via targeted resequencing using amplicon target amplification assays. Validation of mutations by Sanger sequencing. Serum GRN protein measurements via ELISA (Adipogen). Nonsense-mediated mRNA decay analysis via cycloheximide treatment of a lymphoblast cell line, extraction of total RNA, cDNA synthesis and Sanger sequencing.

Results: We identified the GRN p.E393A mutation in a patient with a diagnosis of non-fluent primary progressive aphasia. Neuropathological examination indicated Alzheimer’s dementia pathology and FTLD TDP type A pathology, characteristic for GRN-related FTLD. The mutation is in the last codon of exon 10 which is part of the splice donor sequence. Cycloheximide treatment of lymphoblast cells of a family member carrier pointed to nonsense-mediated mRNA decay of the mutant transcript. GRN protein levels were reduced in serum of the same carrier to a level comparable to other known LOF mutations.

Conclusions: We provide evidence that GRN p.E393A in exon 10 causes aberrant splicing, leading to a frameshift (p.E393fs), degradation of the mutant transcript, reduced GRN serum levels and haploinsufficiency. These results demonstrate that careful examination of resequencing data is mandatory, as this mutation was annotated as a missense mutation.
ON-DEMAND SYMPOSIUM: TDP43- AND C9ORF72-RELATED DISEASES 2

TRODUSQUEMINE PREVENTS TDP-43 INCLUSION FORMATION AND SUPPRESSES ITS TOXICITY IN VITRO AND IN VIVO

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Aims: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease which is characterized by the progressive degeneration of motor neurons in primary motor cortex, brainstem and spinal cord. In 2006 TDP-43 was identified as a key component of the insoluble and ubiquitinated inclusions in the brains of patients suffering from ALS. The pathobiological feature of TDP-43 in ALS is represented by its aberrant cytoplasmic aggregation concomitantly with its nuclear depletion. In the last years, natural molecules such as aminosterols (in particular squalamine and trodusquemine) showed significant protective effects against the pathological aggregation and toxicity of α-synuclein and amyloid-β peptide.

Methods: Here, we evaluated the protective effect of trodusquemine against TDP-43 aggregation and toxicity both in neuronal cells and in a C. elegans model, taking advantage of the high resolution power of Stimulated Emission Depletion (STED) microscopy.

Results: Trodusquemine can prevent the mislocalisation of overexpressed human wild-type full-length TDP-43 in our cell models. In particular, trodusquemine can decrease the cytoplasmic TDP-43 accumulation with a re-localization of the protein in the nucleus and rescue TDP-43 cytotoxicity. When TDP-1 worms were exposed to trodusquemine from L4 larval stage, we observed a decrease in inclusion formation and a very significantly improved motility, with respect to TDP-1 worms in the absence of trodusquemine.

Conclusions: This study provides evidence that trodusquemine could be rationally optimized in drug discovery programs to target TDP-43 toxicity in ALS and other neurodegenerative diseases. The study was supported by Fondazione Cassa di Risparmio di Firenze (TROTHERALS to C.C.).
Aims: Fronto-Temporal Dementia (FTD) is characterized by aggregation of proteins (TDP-43 and Tau) in the frontal and temporal lobes with microvacuolation and relevant deregulation of RNA-binding proteins (RBPs). We investigated miRNA cargo of small Extracellular Vesicles (SEVs) derived from plasma of FTD patients and healthy controls. The purpose was to evaluate deregulated microRNAs in patients to identify new peripheral biomarkers. Moreover, we aimed to identify mRNA targets involved in FTD pathogenesis.

Methods: SEVs were isolated from plasma of 9 FTD patients and 9 healthy volunteers by differential centrifugation and characterized by Nanosight. MicroRNA libraries were generated using Small RNA-Seq Library Prep Kit (Lexogen) and sequenced on a NextSeq 500 (Illumina). Interaction prediction was carried out on TarBase v.8 database.

Results: We found a total of 197 Differentially Expressed microRNAs, 99 up-regulated and 98 downregulated. Then, we looked for directly validated mRNA targets of the most deregulated microRNAs in our analysis. Interestingly, hsa-miR-522-5p, down-regulated in our profiling, targets RTN3 that in turn interacts with and modulates BACE1, and the up-regulation of hsa-miR-203a-3p may impact on TNF and IL-12 levels. Moreover, hsa-miR-181c-5p was up-regulated and its role was already linked to a negative feedback network of TDP43. We also found a down-regulated microRNA in common with Alzheimer’s disease, hsa-miR-1260b, involved in Wnt pathway.

Conclusions: In conclusion, our data highlight the importance of microRNAs cargo examination in EVs of FTD patients. In fact, their potential is exploitable both for biomarkers discovery and for study of gene expression alteration in FTD pathogenesis.
Aims: Intracellular aggregation of TDP-43 is found in most patients with amyotrophic lateral sclerosis (ALS), in 45% of patients with frontotemporal dementia (FTD) and in 30-50% of patients with clinical diagnosis of Alzheimer’s disease. Direct detection of TDP-43 aggregates by positron emission tomography (PET) holds promise for diagnosis, staging, patient stratification, longitudinal measurement of disease progression and assessment of therapeutic efficacy in clinical trials.

Methods: Binding affinities of small molecules from AC Immune’s proprietary Morphomer™ library were evaluated on TDP-43 aggregates enriched from FTLD-TDP brain samples. Target engagement was further assessed by high-resolution and classical autoradiography on sections from postmortem brains with different types of FTLD-TDP pathology. Binding selectivity over other aggregation-prone proteins was evaluated in radioligand binding experiments. Pharmacokinetic profile was established in mice and selected 18F radiolabeled compounds were also profiled in non-human primates.

Results: Screening of >500 compounds led to identification of 3 distinct chemical series that bound pathological TDP-43 derived from FTLD-TDP brain samples with nanomolar affinity. Selectivity over amyloid beta, alpha-synuclein and Tau was established for selected compounds. Target engagement on FTLD-TDP Type A and C inclusions was shown by high resolution autoradiography. For some compounds pharmacokinetic properties suitable for first-in-human PET tracer evaluation were established in non-human primates.

Conclusions: We identified for the first time a compound displaying target engagement on FTLD-TDP Type A and C pathology. Medicinal chemistry optimization is ongoing to further improve the affinity and the CNS pharmacokinetic profile for development as PET tracer.
ON-DEMAND SYMPOSIUM: TDP43- AND C9ORF72-RELATED DISEASES 2

SOCIAL COGNITION IN PRIMARY PROGRESSIVE APHASIA

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Aims: To investigate theory of Mind (ToM) abilities in the semantic (svPPA) and non-fluent (nfvPPA) variants of primary progressive aphasia compared to cases of behavioural variant of frontotemporal dementia (bvFTD).

Methods: We recruited 25 PPA (13 svPPA, 12 nfvPPA) and 27 age-, sex-, and education-matched bvFTD patients. Patients underwent a comprehensive neuropsychological assessment including the Story-based Empathy Task (SET), assessing abilities of intention and emotion attribution (IA, EA). Differences in SET global, IA, and EA scores across groups were assessed using ANCOVA-models. Additionally, pathological distributions in each subtest were calculated for every patient according to normative data and compared across groups using Fisher’s exact test.

Results: SET performances did not differ across groups. 22% (26% IA, 19% EA) bvFTD, 50% (42% IA, 25% EA) nfvPPA and 31% (23% IA, 39% EA) svPPA showed SET pathological scores, although these distributions were not different across groups. Analyses did not reveal differences between groups even when bvFTD were compared with PPA patients combined in a single group (N=25).

Conclusions: We observed no differences between bvFTD, svPPA, and nfvPPA in cognitive and affective ToM tasks. These findings suggest the presence of a broader impairment of cognitive abilities in PPA patients, extending to non-language domains even at the early stage. Further investigations need to clarify whether disease duration and language comprehension play a role in the social cognitive deficits of PPA. These findings offer new potential behavioral markers for early diagnosis of FTLD conditions. Supported by: Italian Ministry of Health (GR-2013-02357415); European Research Council (StG-2016_714388_NeuroTRACK).
ON-DEMAND SYMPOSIUM: TDP43- AND C9ORF72-RELATED DISEASES 2

NEUROANATOMY OF FTLD: CORRELATIONS BETWEEN PSYCHIATRIC SYMPTOMS AND WHOLE-BRAIN (CO-)PATHOLOGY


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Aims: Three distinct pathological proteins accumulate throughout the brain and shape the clinical presentation of frontotemporal lobar degeneration (FTLD): TDP-43, tau, and FUS. Beside the main pathological subtypes, co-pathologies are common. The aim of this study is to investigate how the location and burden of (co-)pathology correlate to early psychiatric and behavioural symptoms of FTLD.

Methods: From the Netherlands Brain Bank cohort (2008-2017), we included 87 FTLD brain donors: 46 FTLD-TDP, 34 FTLD-tau, and seven FTLD-FUS. Post-mortem brain tissue was dissected into 20 standard regions and stained for TDP-43, tau, FUS, amyloid-beta, and alpha-synuclein. We used a semi-quantitative visual score to assess the burden of each pathological protein in each brain region. We scored psychiatric and behavioural symptoms in the first three years of disease. Whole-brain clinico-pathological partial correlations were calculated (local FDR threshold = 0.01).

Results: Hallucinations correlated with higher TDP-43 burden in the hippocampal granular layer (R = 0.33), mania with TDP-43 in CA1 (R = 0.35), depression with TDP-43 in CA3 and with parahippocampal tau (R = 0.30 and R = 0.23), and delusions with CA3 tau (R = 0.26). Disinhibition showed positive correlations with amyloid-beta in the subthalamus (R = 0.24) and alpha-synuclein in the substantia nigra (R = 0.24). Depression correlated with tau pathology in the substantia nigra (R = 0.25) and in the locus coeruleus (R = 0.26).

Conclusions: Psychiatric symptoms of FTLD are linked to subcortical pathology burden in the hippocampus and in the brainstem. Co-occurring non-FTLD pathologies in subcortical regions could shape the clinical presentation of FTLD.
ON-DEMAND SYMPOSIUM: PD, MSA DISEASE MECHANISMS, DIAGNOSIS BIOMARKERS, MICROBIOME

NON-MOTOR SYMPTOMS IN PARKINSON’S DISEASE: A SYSTEMATIC REVIEW AND META-ANALYSIS OF PREVALENCE

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Aims: Most classically identified non-motor symptoms of Parkinson’s disease (PD) are very common in older adults without PD. Identifying non-motor symptoms specific to PD is key to testing the efficacy of novel drugs acting on the core disease biology. We compared the prevalence of non-motor symptoms in individuals with PD with age-matched healthy controls (HCs) to identify those most specific to PD.

Methods: We performed a systematic review and meta-analysis of Embase, SciSearch, MEDLINE and BIOSIS from database inception to 15 March 2021. We included observational studies reporting the prevalence of non-motor symptoms using the non-motor symptom questionnaire or non-motor symptom scale in both individuals with PD and HCs. Using a random effects model, we generated a pooled estimate for the prevalence risk ratio (RR) for each symptom.

Results: A total of 14 studies including 7,243 individuals with PD and 2,592 HCs were identified and included in this meta-analysis. 27 out of 30 non-motor symptoms were more prevalent in individuals with PD than HCs. Excessive drooling and hallucinations showed the greatest prevalence RR (RR=6.76 [95% confidence interval [CI] 4.82–9.49] and RR=5.43 [95% CI 3.28–8.99], respectively) in individuals with PD compared with HCs.

Conclusions: The majority of non-motor PD symptoms are slightly more common in PD than HCs, with excessive drooling and hallucinations being the most specific to PD. Testing novel drugs acting on core PD biology might not be able to modify the progression of non-specific non-motor symptoms and focusing on non-motor symptoms more specific to PD may increase the signal-to-noise ratio.
ON-DEMAND SYMPOSIUM: PD, MSA DISEASE MECHANISMS, DIAGNOSIS BIOMARKERS, MICROBIOME

ENDOGENOUS PINK1 REGULATES DENDRITIC ARCHITECTURE AND SPINOGENESIS

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Aims: Recessive mutations in the gene for PTEN-induced kinase 1 (PINK1) are linked to Parkinson’s disease (PD) and PD with dementia (PDD). We previously discovered that overexpression of PINK1 promotes dendritic complexity through interaction with valosin containing protein and activation of protein kinase A. Furthermore, treatment with a small molecule inhibitor of endogenous PINK1 degradation confers protection against the severe dendritic retraction elicited by MPP+. To further investigate the role of endogenous PINK1 in regulating neuronal structure and function, we studied primary cortical neurons and brain tissues from Pink1 knockout mice.

Methods: Primary embryonic neuron cultures, electrophysiology, tissue Golgi staining and western blot analyses were used to study changes in cortical neuron architecture in vitro and in vivo.

Results: We found that loss of PINK1 expression results in diminished dendritic complexity, reduced spine density, and altered cortical neuron function.

Conclusions: PINK1 plays a previously underappreciated role in regulating neuronal structure. Future studies are directed at understanding whether these changes are developmental and/or reflect decreased resilience against neurodegenerative stressors, and to define the signaling pathways involved.

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ON-DEMAND SYMPOSIUM: PD, MSA DISEASE MECHANISMS, DIAGNOSIS BIOMARKERS, MICROBIOME

CLUSTERING OF DEFICIENT FAECAL METABOLITES ALIGNS WITH CLUSTERING OF CLINICAL CHARACTERISTICS RELEVANT TO IDIOPATHIC PARKINSONISM

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Aims: Viewing the gut as a strategic aetiopathogenic junctional point, we map potential drivers/mediators of idiopathic parkinsonism (IP).

Methods: We characterised 77 people with diagnosed-IP, 113 without: dietary diaries; exogenous-substance consumption; faecal metabolome (NMR); intestinal inflammation; serum cytokines/chemokines; clinical phenotype. Hierarchical-cluster analyses of metabolites discriminant for IP-status and clinical phenotype were performed.

Results: Longer colon-transit was linked to lower concentrations of faecal short-chain-fatty-acids (SCFAs) and a tryptophan-containing metabolite-cluster. Phenotypic-cluster analysis aggregated colonic-transit with brady/hypokinesia, tremor, sleep-disorder and dysosmia, all being associated with lower ‘tryptophan-cluster’ concentrations. Although SCFAs were lower in IP, association with colonic-transit was only outside IP [mean (95% CI) -24 (-33, -14)% butyric acid/10 transit-markers retained (p=0.001)]. However, colonic-transit and its motor/non-motor associates were individually associated with lower tryptophan-cluster concentrations, irrespective of IP-status. Rigidity was isolated in the phenotype-clustering and independent of metabolome. Faster pulse was associated with lower concentrations in a metabolite-cluster containing benzoic acid (anti-fungal) and niacin (anti-inflammatory) and higher serum chemokine CCL20 (chemotactic for lymphocytes/dendritic cells towards epithelium). The faster pulse of IP was irrespective of postural fall in blood pressure. Intestinal inflammation (faecal calprotectin) was greater in IP [44 (5, 98)% (p=0.001, adjusted for proton-pump-inhibitors, anti-microbials]. Free-sugar intake was increased in IP (p=0.001), a higher intake being associated with exceeding inflammatory bowel disease calprotectin cut-point and with lower tryptophan-cluster concentrations. Lower caffeine, alcohol and water intakes (typical of IP) related to lower niacin and benzoic acid concentrations.

Conclusions: IP may be a forme fruste of systemic-inflammatory-response, consequent on intestinal inflammation and faecal metabolome deficits.
Aims: In Parkinson's Disease (PD), several evidence indicates the involvement of gut-brain axis as one of the primary physio-pathological mechanisms underlying α-Syn aggregation and following propagation to CNS. Furthermore, gastrointestinal dysfunctions represent one of the main non-motor symptoms in PD, often preceding the development of proper motor symptoms.

Methods: Our aim was to investigate α-Syn seeding activity in stomach-duodenum biopsies of PD patients by real-time quaking induced conversion (RT-QuIC) assay. The α-Syn RT-QuIC assay follow the seeded aggregation of monomeric αSyn into amyloid fibrils upon seeding by traces of α-Syn aggregates present in biospecimens. 16 patients with symptomatic PD which underwent Duodopa Percutaneous Endoscopic Gastrostomy and Jejunal Tube (PEG-J) procedure were included in the study. A mean of 3 (2mm3) wall biopsies were sampled from each patient, homogenized, and analyzed by RT-QuIC assay. Control biopsies were included from 10 age-and-sex-matched patients undergoing routine diagnostic endoscopy.

Results: We found a relevant α-Syn seeding activity in almost all biopsies of PD patients, with higher response in the stomach biopsies than duodenum and no activity were detected in the control biopsies. α-Syn seeding activity were also detected in all patient's skin biopsy through RT-QuIC.

Conclusions: Enteric nervous system could be one of the earliest implicated structures in the processes of α-Syn aggregation and an unmet clinical need is a reliable diagnostic biomarker in the prodromic phases of disease. We would suggest a deeper investigation in detection of α-Syn seeding activity in the early stages of PD in gastrointestinal biopsies via RT-QuIC as an early biomarker of disease.
ON-DEMAND SYMPOSIUM: PD, MSA DISEASE MECHANISMS, DIAGNOSIS BIOMARKERS, MICROBIOME

DETECTION OF BACTERIAL PROTEINS IDENTIFIED IN BRAIN TISSUE FROM NORMAL AGING AND ALZHEIMER’S DISEASE

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Aims: Alzheimer’s disease (AD) continues to be a major health issue in the USA. Unfortunately, recent clinical trials targeting major pathological hallmarks failed. Therefore, it is imperative to better understand other molecular processes that modulate brain function and health. Several studies suggest that dysbiosis may be associated with AD, but there is no evidence for a causal effect. We aim to detect bacterial proteins in brain tissue from normal aging and Alzheimer’s disease.

Methods: We developed an unbiased proteomics and stringent datamining approach to identify bacterial proteins in normal aging and AD brain tissue from two different brain banks. After the protein list was curated to exclude human orthologues, we identified bacterial proteins corresponding to specific bacterial species in human frontal cortex.

Results: We identified bacterial proteins from 24 bacterial species in both normal aging and AD and 6 bacterial species only in normal aging. Seven out of the 31 bacterial species unique to AD were found in all AD samples from both brain banks and the rest were identified in, at least, five out of six samples.

Conclusions: The results demonstrate that bacterial proteins can be identified in human brain tissue and specific bacterial species are associated with normal aging and AD. Further studies are required to elucidate the effect that the identified bacterial species have on human health and disease.
Aims: The evolutionarily conserved Retromer complex (Vps35-Vps26-Vps29) is essential for endosomal membrane trafficking and signalling and dysfunction is linked to several neurodegenerative diseases including Parkinson's disease, Alzheimer's disease and ALS. We are seeking new molecular tools to modulate Retromer function that could provide new avenues in understanding its function and targeting in associated diseases.

Methods: We have employed the random nonstandard peptides integrated discovery (RaPID) approach to identify a group of macrocyclic peptides capable of binding to Retromer with high affinity. Using biochemical, biophysical, structural and cellular approaches we have characterised their mechanism of action and activity in various binding and functional assays.

Results: Macrocyclic peptides have been identified that bind Retromer with high affinity that can either inhibit its function or as a molecular stabilizer. Inhibitor macrocyclic peptides bind to Vps29 via a dipeptide Pro-Leu sequence. These structurally mimic known interacting proteins including TBC1D5, and VARP, and potent inhibit their interaction with Retromer in vitro and in cells. For the molecular stabilizer, we found that this macrocyclic peptide binds Retromer at the Vps35 and Vps26 interface, and it can act as a molecular chaperone to stabilise the complex with minimal disruptive effects on Retromer’s ability to interact with its accessory proteins.

Conclusions: The macrocyclic peptides identified in this study can be used as a novel toolbox for the study of Retromer-mediated endosomal trafficking, highlight the importance of Vps29 in binding to multiple different accessory proteins and suggest a novel site for targeting Retromer with small molecule chaperones with therapeutic potential in neurodegenerative diseases.
ON-DEMAND SYMPOSIUM: PD, MSA DISEASE MECHANISMS, DIAGNOSIS BIOMARKERS, MICROBIOME

NOVEL PEPTIDIC SCAFFOLDS TO BIND ALPHA-SYNUCLEIN TOXIC SPECIES WITH NANOMOLAR AFFINITY

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Aims: α-Synuclein aggregation is a key driver of neurodegeneration in Parkinson’s disease and related syndromes. Accordingly, obtaining a molecule that targets α-synuclein toxic assemblies with high affinity and conformational selectivity is a long-pursued objective.

Methods: Here, we have exploited the biophysical properties of toxic oligomers and amyloid fibrils to identify a family of α-helical peptides that bind to these α-synuclein species with low nanomolar affinity, without interfering with the monomeric functional protein. This activity is translated into an unprecedented anti-aggregation potency and the ability to abrogate oligomer-induced cell damage.

Results: With a structure-function relationship in hand, we identified a human peptide expressed in the brain and the gastrointestinal tract with exceptional binding, anti-aggregation, and detoxifying properties.

Conclusions: The chemical entities we describe here represent a new therapeutic paradigm and are privileged tools to assist diagnosis by detecting α-synuclein toxic species in biofluids.
ON-DEMAND SYMPOSIUM: PD, MSA DISEASE MECHANISMS, DIAGNOSIS BIOMARKERS, MICROBIOME

A NOVEL STRAIN SPECIFIC SEEDING ASSAY ENABLES THE EVALUATION OF ALPHA-SYNUCLEIN IN MULTIPLE SYSTEM ATROPHY

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Aims: Several in vitro and in vivo findings have consistently shown that α-synuclein derived from multiple system atrophy (MSA) subjects has more seeding capacity than Parkinson’s disease (PD) derived α-synuclein. However, reliable detection of α-synuclein derived from MSA using seeded amplification assays, such as the Real-Time Quaking-induced Conversion (RT-QuIC), has remained challenging.

Methods: We have conducted a systematic evaluation of 168 different reaction buffers, using an array of pH and salts, seeded with fully characterized brain homogenates from one MSA and one PD patient. Using this original approach, we have been able to categorize 15 MSA brains according to their α-synuclein seeding behavior. We have also conducted the first multi-regional evaluation of the α-synuclein seeding in 13 different regions from six MSA brains.

Results: Here we demonstrate that the interaction of the Thioflavin T dye with α-synuclein from MSA and PD patients can be modulated by the type of salt, pH, and ionic strength used to generate strain-specific reaction buffers. Employing this novel approach, we have generated a streamlined RT-QuIC assay capable of categorizing MSA brains according to their α-synuclein seeding behavior, and to unravel a previously unrecognized heterogeneity in seeding activity between different brain regions of a given individual that goes beyond immunohistochemical observations.

Conclusions: Our observations pave the way for future subclassification of MSA, that should go beyond the conventional clinical and neuropathological phenotyping and consider the structural and biochemical heterogeneity of α-synuclein present in these patients. Finally, our results provide the experimental framework for the development of novel diagnostic assays for MSA.
Rapid oligomerization of alpha synuclein at mitochondria induces neuronal toxicity in Parkinson’s disease

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Aims: Aggregation of α-synuclein drives Parkinson’s disease, although the initial stages of self-assembly and structural conversion have not been captured inside neurons. We aim to track live the kinetics, triggers, and location of α-synuclein aggregation inside rodent and human neurons to investigate how protein-lipid interactions may trigger aggregation and alter the kinetics of aggregation in Parkinson’s disease.

Methods: Our study integrated high-resolution biophysical approaches utilizing a single-molecule Förster resonance energy transfer (smFRET) biosensor and 3D FRET - Correlative light and electron microscopy (CLEM) with human iPSC biology to comprehensively characterize the spatial and temporal features of protein aggregation in the intracellular environment.

Results: We show that α-synuclein converts from its monomeric state to form two distinct oligomeric states in neurons in a concentration-dependent and sequence-specific manner. 3D FRET- CLEM reveals the structural organization and location of aggregation hotspots inside the cell. Notably, multiple intracellular seeding events occur preferentially on membrane surfaces, especially mitochondrial membranes. The mitochondrial lipid cardiolipin triggers rapid oligomerization of A53T α-synuclein, and cardiolipin is sequestered within aggregating lipid-protein complexes. Mitochondrial aggregates impair complex I activity and increase mitochondrial ROS generation, which accelerates the oligomerization of A53T α-synuclein, and ultimately causes permeabilization of mitochondrial membranes and cell death. Patient iPSC derived neurons harboring A53T mutations exhibit accelerated oligomerization that is dependent on mitochondrial ROS, early mitochondrial permeabilization and neuronal death.

Conclusions: Our study highlights a mechanism of de novo oligomerization at the mitochondria and its induction of neuronal toxicity.
Aims: A variety of therapeutic strategies failed in multiple system atrophy (MSA), an atypical parkinsonian disorder characterized by a fast disease progression, emphasizing the urgent need for the development of novel therapeutic approaches. Neuropathological hallmarks of MSA include alpha-synuclein inclusions within oligodendrocytes, myelin deficit and a severe, region-specific neuroinflammation. In MSA patients and its corresponding mouse model (MBP29) early increase of myeloid cell numbers, proliferation, and activation has been observed. Thus, colony-stimulating factor 1 receptor-dependent myeloid cell depletion using the small-molecule inhibitor (PLX5622) may improve motor function and neuropathology in MBP29 mice.

Methods: Animals were treated with 1200 mg/kg PLX5622 supplemented in chow or control food for 3 months. Assessment of motor functions and structural analysis was performed using histological and biochemical approaches at 16 weeks of age.

Results: We observed premature death of 25% of control-treated MBP29 mice, while 100% of PLX5622-treated MBP29 mice survived. Furthermore, a delayed onset and reduced frequency of neurological symptoms was observed upon PLX5622 treatment. Surprisingly, assessment of motor function showed a severe impairment of MBP29 mice upon PLX5622 treatment. Histological and biological analysis revealed myeloid cell depletion by up to 99%, however, no recovery of myelin nor decrease of alpha-synuclein levels. Interestingly, depletion of myeloid cells reduced the density of dopaminergic neurons in the substantia nigra pars compacta, the prototypical region important for parkinsonian motor deficits.

Conclusions: Our results identify that widespread myeloid cell depletion shows diverse functions in the present MSA model improving life span at the expense of motor functions.
ON-DEMAND SYMPOSIUM: AD ANIMAL MODELS AND MECHANISTIC & TRANSLATIONAL ASPECTS 2

INVESTIGATIONS ON GENE EXPRESSION SIGNATURES AND AD-LIKE PATHOLOGY IN NOVEL HUMAN TAU KNOCK-IN MICE UNDER PROGRESSING CEREBRAL AMYLOIDOSIS

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Aims: Modeling Alzheimer’s disease in mice is key for untangling the contribution of genes involved in disease development. Here, we studied the effects of fast-progressing amyloidosis in novel human tau knock-in mice crossbred with 5xFAD mice, creating 5xFADxhtau-KI mice.

Methods: Brain gene expression of 5xFADxhtau-KI mice was analyzed and compared with that in human AD brain regions, applying the nCounter Mouse AD gene expression panel (Nanostring). We further characterized AD-like pathology by biochemical and immunohistochemical methods, analyzed synaptic transmission and plasticity by LTP measurements and single cell patch-clamp and monitored the behavior of these mice.

Results: The results from the multiple linear regression analysis revealed distinct effects between the 5xFAD and htau-KI mouse models when correlated to 30 human co-expression modules derived from seven brain regions. Conspicuous changes were identified in pathways related to mitochondria biology, extracellular matrix- and immune function. Observation at the transcriptional level were corroborated by electrophysiology and histopathology. LTP deficits were noted in 5xFAD and htau-KI mice in contrast to signs of rescue in 5xFADxhtau-KI mice. Increased frequencies of miniEPSCs and miniIPSCs indicated an upregulated presynaptic function in 5xFADxhtau-KI. Furthermore, these changes were accompanied by plaque-associated MC1-positive pathological tau that was exclusively observed in 5xFADxhtau-KI mice

Conclusions: The found physiological levels of human tau protein in 5xFADxhtau-KI mice interfere differentially with progressing levels of Aβ than endogenous murine tau in 5xFAD mice. This indicates the activation of distinct AD-related molecular pathways under both conditions with implications for future model development in AD research.
ON-DEMAND SYMPOSIUM: AD ANIMAL MODELS AND MECHANISTIC & TRANSLATIONAL ASPECTS 2

MOUSE MODELS FOR IN VIVO TESTING OF GENETIC RISK FACTORS FOR LATE-ONSET ALZHEIMER'S DISEASE

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Aims: While numerous genetic risk factors for late-onset Alzheimer's disease (LOAD) have been identified, few have been experimentally verified in vivo. The field is lacking in animal models to study mechanisms of LOAD genetic risk and for preclinical testing of potential therapeutics. The Model Organism Development and Evaluation for Late-onset AD (MODEL-AD) Consortium was established to generate and characterize more translatable animal models for LOAD based on human genetic risk variants.

Methods: Both coding and non-coding LOAD risk variants were prioritized based on human data sets. These alleles were engineered into mouse models expressing humanized APOE4 and the Trem2*R47H risk variant. A novel transcriptomic panel based on clinical LOAD samples (Preuss et al, 2020) was used at 4 and 12 months of age to evaluate how well these models replicated clinical transcriptomic changes with disease. This was used as a primary screen to select models for detailed characterization using clinically relevant measures including various 'omics, biomarkers, neuropathology and in vivo imaging at advanced ages.

Results: We identified discrete human AD-related pathways that were disrupted in novel mouse models in an age-dependent manner. Specifically, mouse models carrying human AD risk variants in Abca7, Mthfr, and Plcg2 mouse genes showed similar changes as those seen in LOAD patients.

Conclusions: We have prioritized mouse models expressing LOAD risk variants in Abca7, Mthfr, and Plcg2 for comprehensive phenotyping at advanced ages. We are currently phenotyping novel models expressing human risk variants in non-coding regions of the Bin1 and Adamts4 loci.
ON-DEMAND SYMPOSIUM: AD ANIMAL MODELS AND MECHANISTIC & TRANSLATIONAL ASPECTS 2

P53-DEPENDENT NEUROINFLAMMATORY RESPONSE TO BETA-AMYLOID CONTRIBUTES TO NEURODEGENERATION AND MEMORY IMPAIRMENT IN AN ANIMAL MODEL OF ALZHEIMER’S DISEASE

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Aims: Amyloid-beta (Aβ) and Tau protein aggregates lead to neuronal loss and cognitive decline in Alzheimer’s Disease (AD) patients. We have previously described a key role for p53 in Aβ-induced neurodegeneration. Recently, glia-induced neuroinflammation has emerged as a key factor in AD pathophysiology and p53 has been postulated as a modulator of the immune response in microglia. Here, we evaluated the possible role of p53 as a regulator of the microglial response to Aβ, which contributes to neurodegeneration.

Methods: Oligomerized Aβ25-35 (9 nmol) was intracerebroventricularly injected into wild-type (WT) and p53 knockout (p53KO) mice. Some WT animals were treated intraperitoneally with the p53 transcriptional activity inhibitor, pifithrin-alpha (PFT-α; 2 mg/kg). Neuroinflammation and neurodegeneration were assessed up to 5 days after injection by Western blot and immunofluorescence. Mice cognitive status was evaluated using NOR, Y-Maze and Barnes Maze tests.

Results: We found that Aβ25-35 oligomers caused p53 accumulation, leading to rapid microglial activation. Also, a switch from M1 (proinflammatory/neurotoxic) to M2 (anti-inflammatory/neuroprotective) microglial profile happened in a p53-dependent manner. Moreover, Aβ25-35 injection also induced reactive astrogliosis. Together, these events led to neurodegeneration and memory impairment, which were prevented by genetic and pharmacological inhibition of p53.

Conclusions: Our results demonstrate a key role of p53 in the Aβ-induced inflammatory response, which may contribute to neurodegeneration and cognitive impairment in AD. Funded by Instituto de Salud Carlos III (PI18/00265; RD16/0019/0018); FEDER; Junta de Castilla y León (CSI151P20; co-financed with FEDER funds), European Union’s Horizon 2020 Research and Innovation Programme (Grant Agreement 686009).
ON-DEMAND SYMPOSIUM: AD ANIMAL MODELS AND MECHANISTIC & TRANSLATIONAL ASPECTS 2

SPATIOTEMPORAL CHARACTERIZATION OF GLIAL CELL ACTIVATION IN AN ALZHEIMER’S DISEASE MODEL BY SPATIALLY RESOLVED TRANSCRIPTOME

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Aims: The pathophysiological changes that occur with the progression of Alzheimer’s disease (AD) are well known, but understanding the spatiotemporal heterogeneity of the brain is needed. We investigated the spatially resolved transcriptome in a 5XFAD AD model of different ages to understand regional changes at the molecular level.

Methods: Spatially resolved transcriptome data were obtained from 5XFAD AD models and control mice. Differentially expressed genes in spots clustered according to anatomical structures were identified. Gene signatures of activation of microglia and astrocytes were mapped on the spatially resolved transcriptome data. Molecular features associated with the co-registered image of amyloid plaques were analyzed by integrating image features with gene expression. To identify spatiotemporal patterns of microglial activation, trajectory inference using a gene set of microglial signature was performed.

Results: We identified early alterations in the white matter (WM) before the definite accumulation of amyloid plaques in the gray matter (GM). Changes in the early stage of the disease were involved primarily in glial cell activation in WM, whereas the changes were prominent in the later stage of pathology in GM. We confirmed that disease-associated microglia (DAM) and astrocyte (DAA) signatures also showed initial changes in WM and that activation spreads to GM. Trajectory inference using microglial gene sets revealed the subdivision of DAMs with different spatial patterns.

Conclusions: Taken together, these results help to understand the spatiotemporal changes associated with reactive glial cells as a major pathophysiology of AD and provide information for diagnosis and prognosis based on spatiotemporal changes caused by amyloid accumulation in AD.
ON-DEMAND SYMPOSIUM: AD ANIMAL MODELS AND MECHANISTIC & TRANSLATIONAL ASPECTS 2

DISTRIBUTION OF TAU PHOSPHORYLATION AT SEVERAL RESIDUES AFTER INJECTION OF PATIENT-DERIVED SEEDS IN A TAU MOUSE MODEL

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Aims: Tauopathies are a heterogeneous class of neurodegenerative diseases, including Alzheimer’s disease (AD), which are characterized by intracellular inclusions of aggregated tau protein. Mirroring the disease by injecting human patient-derived tau into a mouse brain is currently an active research field as it recapitulates the cell type specificity and structural features of tau aggregation of the donors tauopathy. Here we evaluated seeding efficacy and phosphorylation pattern of tau in the hTau mouse model.

Methods: hTau transgenic mice, developed by K. Duff and colleagues, express human tau on a murine tau knockout background. Mice were injected with tau seeds isolated from human AD brains (AD-tau seeds) or vehicle into the right dorsal hippocampus and overlying cortex. After 12 weeks, brain tissue was harvested and coronal sections were collected from the injection site. Sections were immunofluorescently labeled for tau phosphorylation at residues tyrosine 18, serine 202/threonine 205, threonine 231, and serine 396 as well as NeuN and GFAP which were subsequently evaluated qualitatively and quantitatively.

Results: Phosphorylation was increased at all analyzed tau residues in the injected area compared to the contralateral hemisphere. Intriguingly, also spreading of tau protein to the contralateral site was detected. Although the granule cell layer of the dentate gyrus was structurally affected by the AD-tau seeds, no neuronal loss was observed. Astrogliosis was evident in the hippocampus after injection of AD-tau seeds.

Conclusions: The presented in vivo tau seeding mouse model is suitable to study tau pathology as well as the efficacy of new therapeutic interventions.
DISTINCT CONTRIBUTIONS OF PARVALBUMIN AND SOMATOSTATIN INTERNEURONS TO COGNITIVE RESILIENCE AND DECLINE IN ALZHEIMER’S DISEASE

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Aims: Alzheimer’s disease patients exhibit variability in the impact of β-amyloid and tau on cognitive decline, with greater resilience to pathology associating with higher cognitive reserve. However, the underlying mechanisms contributing to cognitive reserve are not fully understood and often underrepresented in preclinical models. Herein, we utilized the TgF344-AD rat model, which exhibit tau pathology from endogenously-expressed tau, and explored the hypothesis that GABAergic neuroplasticity underlies cognitive resilience.

Methods: At 9-, 12- and 15-months, TgF344-AD and non-transgenic rats underwent pathological analyses, including quantification of β-amyloid plaques, tau inclusions, and neurons. Somatostatin and parvalbumin interneurons were assessed for vulnerability to tau and traced to examine potential neuroplasticity. Finally, cognition was tested in the Barnes maze.

Results: Excitatory and inhibitory neurons were lost in the entorhinal cortex and hippocampus of 9-month-old TgF344-AD rats. Interestingly, hippocampal neuronal compensation consisting of upregulated GABAergic markers occurred in 12-month-old TgF344-AD rats, before robust loss at 15-months. β-amyloid and tau accumulated continually across age in TgF344-AD rats. Hippocampal somatostatin and parvalbumin GABAergic subtypes demonstrate differing susceptibility to pathology, with somatostatin intracellular tau inclusions and neurodegeneration from 9-months, and parvalbumin resilience until 15-months of age. The TgF344-AD hippocampal parvalbumin circuit underwent neuroplastic reorganization at 9- and 12-months, before robust degeneration at 15-months. Strikingly, a stage of cognitive resilience in executive function and cognitive flexibility occurred in 12-month-old TgF344-AD rats, with significant impairment at 9- and 15-months; yet progressive spatial memory deficits.

Conclusions: Our results demonstrate that GABAergic compensation, including parvalbumin resilience and neuroplasticity, sustain cognition in Alzheimer’s disease.
H4K20ME1-MEDIATED EPIGENETIC DYSREGULATION OF MTOR/AUTOPHAGY INCREASES AMYLOID BETA ACCUMULATION AND COGNITIVE/NEUROMOTOR DEFICITS IN HYPERHOMOCYSTEINEMIC AND BLEOMYCIN HYDROLASE-DEFICIENT MICE

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Aims: The mammalian target of rapamycin (mTOR) is epigenetically regulated by H4K20me1 and histone demethylase Phf8. Hyperhomocysteinemia (HHcy), alterations in mTOR/autophagy and bleomycin hydrolase gene (BLMH) are linked to Alzheimer’s disease, but underlying mechanisms are not fully understood. We tested a hypothesis that epigenetic dysregulation of mTOR/autophagy by HHcy causes amyloid-beta (Aβ) accumulation and neurological impairment.

Methods: We used Aβ-overexpressing Blmh⁻/⁻5xFAD mice and N2A-APPswe neuroblastoma cells. HHcy was induced by supplementation with Met (mice) or homocysteine (cells). Hcy-thiolactone levels in cells were manipulated by siRNA silencing of Blmh gene or supplementation with Hcy-thiolactone. Aβ, mTOR/autophagy proteins, and mTOR gene occupancy by Phf8/H4K20me1 were quantified by confocal microscopy, Western blotting, and chromatin immunoprecipitation (Chip) assays, respectively. Neurological impairments were assessed by behavioral testing.

Results: Blmh⁺/+5xFAD and Blmh⁻/⁻5xFAD mice fed with an HHcy diet showed significantly impaired neuro-motor activity (beaker test) and cognition (Novel Object Recognition test), relative to non-HHcy Blmh⁺/+5xFAD animals. Brains of HHcy mice showed significantly elevated Aβ, phospho-mTOR, and decreased autophagy markers, compared to Blmh⁺/+5xFAD non-HHcy controls. Phf8 was decreased and H4K20me1 increased in brains of HHcy Blmh⁺/+5xFAD and Blmh⁻/⁻5xFAD mice, compared to Blmh⁺/+5xFAD non-HHcy controls. Similar changes were observed in N2A-APPswe cells after silencing Blmh gene or treatments with Hcy or HTL. Chip assay showed increased occupancy of mTOR promoter by H4K20me1 in Hcy/HTL-treated N2A-APPswe cells.

Conclusions: Blmh deficiency and HHcy dysregulated mTOR signaling/autophagy via increased mTOR promoter occupancy by Phf8-dependent H4K20me1, which caused Aβ accumulation and ultimately impaired cognition and neuro-motor activities. Supported by NCN grants 2016/21/D/NZ4/00478, 2016/23/N/NZ3/01216, 2018/29/B/NZ4/00771, 2019/33/B/NZ4/01760.
ON-DEMAND SYMPOSIUM: AD ANIMAL MODELS AND MECHANISTIC & TRANSLATIONAL ASPECTS 2

EARLY ACTIVATION OF TOLL-LIKE RECEPTOR-3 REDUCES ATTENUATES PATHOLOGICAL PROGRESSION AND IMPROVES NEUROBEHAVIORAL FUNCTIONS IN APP/PS1 MOUSE MODEL OF ALZHEIMER’S DISEASE

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Aims: Toll-like receptor 3 (TLR3), as a natural immune receptor, plays an important role in the activation and regulation of immune/inflammatory response in nervous system, which is the main pathological features of Alzheimer’s disease (AD). The present study is to investigate the role of TLR3 in the pathophysiological process and neurobehavioral dysfunction of AD.

Methods: In the experiment, agonist of TLR3, Poly(I:C), was intraperitoneally injected into the APP/PS1 mouse model of AD and wild type (WT) control mice starting from the age of 4 months to 9 months. At the age of 14 months, behavioral tests were detected. After that, Western blots and immunohistochemistry (IHC) staining were used to evaluate the level of Amyloid β protein (Aβ), activation of inflammatory cells, and neuron loss. In addition, levels of inflammatory cytokines were measured using quantitative PCR (qPCR).

Results: The results demonstrated that early activation of TLR3 attenuated neuronal loss and neurobehavioral dysfunction. Moreover, early-activation of TLR3 reduced Aβ protein deposition, inhibited the activation of microglia and astrocytes, and decreased the transcription of pro-inflammatory factors in hippocampus.

Conclusions: The results indicated that early activation of TLR3 mediated signaling reduced the pathological progression and neurobehavioral dysfunction in mouse model AD.
ON-DEMAND SYMPOSIUM: COVID-19 AND OTHER VIRUSES: IMPACT ON NEURODEGENERATIVE DISEASES

PROTEOMIC PROFILING IDENTIFIED PLASMA BIOMARKERS FOR SARS-COV-2 INFECTION AND SEVERITY OF COVID-19 PATIENTS

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Aims: Coronavirus disease 2019 (COVID-19) has infected over 128 million people leading to over 4 million deaths worldwide. Identifying biomarkers for COVID-19 infection and severity is critical for understanding the pathogenesis of this disease and developing new therapeutic approach.

Methods: We performed differential analyses of log10-transformed 7055 protein levels with each of the three outcomes related to COVID-19: infection status, ventilation, and mortality using 332 COVID-19 patients and 150 controls recruited at Washington University. For external replication, we obtained and analyzed 4301 proteins levels for 306 COVID-19 cases and 78 controls recruited at Massachusetts General Hospital.

Results: We identified 3024 significant proteins for infection status (1057 replicated), 1158 for ventilation (655 replicated), and 806 for mortality (461 replicated). Forty-four proteins were significant and consistent across all three outcomes. They were a part of “500 genes up-regulated by SARS-CoV-2 in human A549 cells from GSE154613” (FDR=6.56x10⁻⁵). Enrichment analyses highlighted several pathways including cytokine-mediated signaling pathway (FDR=3.7x10⁻³) and platelet degranulation (FDR=1.19x10⁻²). Prediction model using these 44 proteins achieved the area under the curve (AUC) ranging from 0.93 to 1 for three outcomes in both discovery and replication cohorts. Interestingly, a biomarker of brain injury NFL was significantly elevated in all three outcomes (P<7.08x10⁻⁷).

Conclusions: Our proteomic analyses identified multiple proteins and pathways associated with COVID-19 infection and severity. The identified pathways include several druggable genes that could be potential targets for intervention. They include IL6, inhibitors of which FDA recently approved for COVID-19. Our findings highlight power of proteomic study for developing therapeutic targets for COVID-19.
ON-DEMAND SYMPOSIUM: COVID-19 AND OTHER VIRUSES: IMPACT ON NEURODEGENERATIVE DISEASES

ROLE OF IL-1Β IN THE SYNAPTIC DYSFUNCTION INDUCED BY RECURRENT HERPES SIMPLEX VIRUS TYPE-1 REACTIVATIONS IN THE ADULT MICE BRAIN

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Aims: A growing body of evidence suggests that HSV-1 infection is a risk factor for Alzheimer’s disease (AD). We recently developed a mouse model of multiple Herpes Simplex Virus Type-1 (HSV-1) replications within the brain showing typical AD hallmarks that include increased proinflammatory cytokine levels. Here, we investigated the contribution of Interleukin 1beta (IL-1β) increment to the HSV-1-induced synaptic dysfunction.

Methods: HSV-1 was inoculated via lip scarification in 1-month-old male C57/Bl6 mice. After two thermal stresses inducing virus reactivation, the AD-like phenotype was studied by electrophysiology, biochemistry, molecular biology and Real-Time PCR.

Results: In HSV-1-infected mice we found increased levels of IL-1β (20.9 ±1.1 vs. 5.8 ±2.1 pg/mg in mock-infected mice, p<0.05) that correlated with synaptic dysfunction assessed by deficits of: i) long-term potentiation (LTP) at CA3-CA1 hippocampal synapses; ii) pre- and post-synaptic proteins; iii) dendritic spine density in hippocampal neurons; iv) expression of plasticity-related genes. These effects were associated with enhanced mRNA levels of two transcriptional repressors, MeCP2 and REST (+5.5±1.4 and +3.0±0.8 fold increase vs. mock-infected mice, respectively; p<0.05). Of note, treatment of infected mice with the interleukin receptor antagonist, anakinra (30 mg/Kg i.p.): i) ameliorated LTP (78.7±12.6 vs. 58.2±7.9% without anakinra, P<0.05); ii) rescued the expression of synaptic proteins and dendritic spine density; iii) counteracted the HSV-1-induced increases in MeCP2 and REST at both mRNA and protein levels.

Conclusions: Our results suggest that IL-1β-activated pathways involving upregulation of MeCP2 and REST contribute to an AD-like phenotype in C57/Bl6 mice subjected to multiple HSV-1 reactivations in the brain.
ON-DEMAND SYMPOSIUM: COVID-19 AND OTHER VIRUSES: IMPACT ON NEURODEGENERATIVE DISEASES

PROBABLE INVOLVEMENT OF VARICELLA ZOSTER VIRUS IN ALZHEIMER’S DISEASE (AD)/DEMENTIA VIA REACTIVATION OF QUIESCENT HERPES SIMPLEX VIRUS TYPE 1

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Aims: Infections are associated with cognitive decline and development of AD/dementia. Epidemiological studies have revealed an increased AD/dementia risk after shingles, caused by varicella zoster virus (VZV), and/or a decreased risk after vaccination against shingles. This suggests that VZV might reside latently in brain, and on reactivation might cause direct damage in brain, as is thought to be the case with herpes simplex virus type 1 (HSV1), a virus strongly implicated in AD. Alternatively, shingles-induced inflammation could lead to neuroinflammation and reactivation of HSV1 in brain, i.e., to an indirect consequence – a suggestion previously made to account for the risk of AD/dementia conferred by infections (Itzhak and Dobson 2002), and for the reduction in risk after certain vaccinations - by preventing occurrence of infection and consequent reactivation. Our aim was to investigate these possibilities by infecting cell cultures with VZV.

Methods: Experiments were carried out infecting two-dimensional human-induced neural stem cells (hiNSC) cultures, and cultures quiescently infected with HSV1 with VZV.

Results: Cells infected with VZV do not show the main AD characteristics - beta amyloid and P-tau accumulation – which HSV1 does cause, but do show gliosis and increased levels of several cytokines, suggesting that VZV’s action is indirect. However, very intriguingly, we found that VZV infection of cells quiescently infected with HSV1 leads to reactivation of HSV1 and consequent AD-like changes, including beta amyloid and P-tau accumulation, supporting the above infection-reactivation concept.

Conclusions: Our results support a role for VZV in AD/dementia via reactivation of HSV1 in brain.
ON-DEMAND SYMPOSIUM: COVID-19 AND OTHER VIRUSES: IMPACT ON NEURODEGENERATIVE DISEASES

EXPLORING SCHIZOPHRENIA (“DEMENTIA PRAECOX”) , ALZHEIMER DEMENTIA (AD) AND COVID-19: CONVERGING ON CARDIO-VASCULAR METABOLIC (CVM) RISKS: POST-HOC ANALYSIS OF GINSANA-115 STUDY IN TREATMENT RESISTANT SCHIZOPHRENIA

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Aims: Meta-analysis finds evidence that Schizophrenia with cognitive impairment carries elevated AD risk. Schizophrenia and AD are adversely affected by COVID-19 in accelerating functional decline, raising the issue whether cardio-vascular-metabolic dysregulation impacts COVID-19 outcome. Pubmed search identifies NLRP3-Inflammasomes-caspase-interleukin cascade as integrative model for aberrant inflammation and immunity landscapes in AD, schizophrenia and COVID-19 cytokine storm. Panax Ginseng regulates inflammasome activation cascade driving immune responses and Tauopathy in AD. Our aim is to examine whether in TRS; 1)Neurocognitive Screen(NCS)index in TRS correlates with Framingham risk scores (FRS), insulin resistance(IR) and lipid profile; 2)Ginsana-115 reduces CVM risks

Methods: We conducted post-hoc analysis of our randomized placebo-controlled 8-week parallel group study of proprietary formulated Ginsana-115(Boehringer-Ingelheim-Pharmaton, Switzerland)

Results: We recruited 35 TRS subjects:mean age 38.0 years). Log-IR scores significantly correlated with spatial processing (p=0.017). FRS correlated inversely with visual perception p = 0.040) and spatial processing (p=0.029) and neurocognitive index (p=0.090). Systolic blood pressure (sBP) correlated significantly and inversely with visual perception (p=0.032),speed (p=0.010),and spatial processing (p=0.034). diastolic blood-pressure correlated with spatial processing (p=0.50). HDL correlated with memory score:p=0.008. Brief-Psychiatric-Rating-Scale: BPRS correlated significantly with executive reasoning ( p=0.047),memory (p=0.021), and mental inflexibility (p=0.026). Ginsanas-115 at 100-mg/200-mg significantly reduced FRS by 24.1% compared with 16.1%(P<0.05) and sBP (P<0.05) and significantly improved lipid profile in raising HDL and lowering LDL.

Conclusions: Our findings are consistent with positive results of Panax Ginseng in AD and schizophrenia, and resetting NLRP3-inflammasome in COVID-19. Panax Ginseng in targeting CVM risks and NLRP3 inflammasomes can be the game-changer in immunity-inflammation-Tau cross talks AD/TRS/COVID therapeutics development.
ON-DEMAND SYMPOSIUM: COVID-19 AND OTHER VIRUSES: IMPACT ON NEURODEGENERATIVE DISEASES

PANDEMIC NEUROLOGICAL AND PSYCHOSOCIAL IMPLICATIONS AND THE GENESIS OF LONG-COVID IN THE ELDERLY: DATA FROM THE ABBIATEGRASSO BRAIN BANK

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Aims: To discover the neuro-behavioral, and psychosocial consequences of COVID-19 and their possible implications in the genesis of long-COVID.

Methods: Study 1: Retrospective analysis of 59 cases with dementia and COVID-19, conducted during the pandemic peak in 2020, to clarify the prognostic significance of delirium. Study 2: Neuropathological and transcriptomic comparison between 9 COVID-19 cases (with and without dementia) and 6 matched non-COVID controls. Study 3: telephone survey during lockdown, conducted on 204 cognitively assessed elderly.

Results: Study 1: Delirium was an onset symptom in 37% of cases and strongly associated with higher mortality (p<0.001), independently associated with an increased risk of mortality (OR:17.0-95% CI:2.8-102.7; p=0.002). Study 2: COVID-19 brains showed nonspecific hypoxic agonic changes, and a variable degree of pre-existing neuro-degeneration. The picture was dominated by hyperactivation of innate immunity (CD68-positive amoeboid microglia), while lymphocytes were scant with minimal antigenic traces of SARS-CoV-2 only in the brainstem, where microglial activation was higher (p=0.046). There were microglia increase in the hippocampus (p=0.048) of cases with delirium. The amount of viral RNA in the frontal cortex was minimal, detectable only by a very sensitive method (dd-PCR). The COVID-19 transcriptional signature in the brain shows reduction of the hypoxia inducing factor, with an increase in IncRNA CTB-36O1.7 (microglial modulator). Study 3: subjects with dementia were less able to adapt, and more depressed. Memory worsening occurred in dementia patients (p=0.006).

Conclusions: Long-COVID in elderly patients is not the result of a direct brain invasion by SARS-CoV-2, but derives from a complex interaction between biological and psychosocial factors.
ON-DEMAND SYMPOSIUM: COVID-19 AND OTHER VIRUSES: IMPACT ON NEURODEGENERATIVE DISEASES

DOES COVID-19 INCREASE THE RISK OF NEUROPSYCHIATRIC AND NEUROLOGICAL SEQUELAE? EVIDENCE FROM MENDELIAN RANDOMIZATION

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Aims: Observational studies based on Electronic Health Records (EHR) reported an increased risk of neurological/neuropsychiatric sequelae for patients affected by Covid-19. However, these studies may suffer from biases like unmeasured confounding, residual reverse causality or lack of precision in EHR-based diagnoses. Here we carried out a mendelian randomization (MR) analysis to Covid-19 susceptibility and different neurological/neuropsychiatric sequelae, so to provide unbiased evidence of potential causal links.

Methods: We applied a two-sample MR analysis to test whether susceptibility to Covid-19 could predispose to an increased risk of different psychiatric/neurodegenerative disorders, including major depression, anxiety, schizophrenia, stroke, Parkinson’s and Alzheimer’s Disease. We analyzed summary statistics from large Genome Wide Association Studies on Covid-19 susceptibility and all the disorders tested, through Inverse Variance Weighted regressions. MR analyses were repeated testing variants associated with three different Covid-19 exposures, namely all (112,612), hospitalized (24,274) and severe cases (8,779) compared to population controls (N>1 million, https://www.covid19hg.org/results/r6/).

Results: MR revealed a significantly increased risk of Alzheimer’s Disease (1-4%) for all the Covid-19 forms tested, with severe and hospitalized forms surviving Bonferroni correction (p < 8.3×10⁻³). Also, we observed a milder association with increased anxiety risk (by 0.5-1%), which survived Bonferroni correction only for hospitalized cases. These findings represent robust evidence suggesting that Covid-19 – particularly the most severe forms – increase the risk of neuropsychiatric sequelae, prominently Alzheimer’s disease, in line with large EHR-based studies.

Conclusions: These results further warrant a targeted screening strategy to tackle the neuropsychiatric post-Covid pandemic.
ON-DEMAND SYMPOSIUM: COVID-19 AND OTHER VIRUSES: IMPACT ON NEURODEGENERATIVE DISEASES

EXPLORING THE BASIS OF COVID-19-RELATED NEUROLOGICAL SEQUELAE: FIRST RESULTS FROM THE SAHLGRENSKA NEUROCOVID STUDY

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Aims: Patients with COVID-19 often report and present with a wide range of neurological symptoms, but the underlying mechanisms have only scarcely been characterised. The Sahlgrenska NeuroCOVID Study aims to map neurological sequelae in COVID-19 patients longitudinally with a focus on multi-domain cognitive impairment and associated processes using an array of modalities.

Methods: We will recruit 20 hospitalised COVID-19 patients (Group 1) who have either been treated with high-flow oxygen, several of which at the ICU (severe disease severity), or with oxygen (moderate), 20 convalescent patients (Group 2) with persisting neurological or cognitive symptoms and 20 age-matched healthy controls (HC). All subjects undergo comprehensive structural and functional MRI and [18F]FDG PET brain imaging, lumbar puncture, blood sampling and thorough neuropsychological examination including testing of olfactory and gustatory function. Group 1 will be examined on four occasions, Group 2 on two and HC on one over the 12-months course of the study.

Results: Preliminary findings from the hitherto recruited 19 patients from Group 1 (mean age 53.6 y, 7 females), 15 from Group 2 (mean age 46.9 y, 8 females) and 20 HC (mean age 53.7 y, 14 females) highlight the prevalence of subjective (subjective cognitive impairment and mental fatigue) and objectively assessed cognitive and psychological sequelae of COVID-19 affecting predominantly executive function, attention and speed domains.

Conclusions: There is a great need to understand the mechanisms underlying the multi-faceted long-term neurological consequences of COVID-19. We will present detailed 6-months data focusing on the relationship between cognitive performance and bodily fluid- and neuroimaging-derived biomarkers.
ON-DEMAND SYMPOSIUM: AD ANIMAL MODELS AND MECHANISTIC & TRANSLATIONAL ASPECTS 3

RESTORATION OF COGNITIVE FUNCTIONS, REDUCTION OF TAU, AMYLOID AND NEUROINFLAMMATION IN VIVO BY MODULATING THE BETA-SECRETASE

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Aims: Amyloid deposition and neurofibrillary degeneration are the pathological processes that define Alzheimer’s disease (AD). We have developed several families of drugs acting both on amyloid and Tau pathologies in vitro and in vivo (Sergeant et al., 2019; Tautou et al., 2021). Through structure-activity relationship, pharmacophore design, and synthesis of novel compounds, we have selected non-competitive β-secretase inhibitors differing by a single azote and having or not a lysosomotropic activity. Our objective was to determine which of the β-secretase or lysosomotropic properties is necessary for beneficial pharmacological effect in vivo.

Methods: Transgenic models of neurodegeneration (Thy-Tau22) and amyloid deposition (APPxPS1) were treated with drug in a curative paradigm. Short and long-term memories, as well as orthogonal biochemical analyses, were used to determine the effect of polyamine biaryl-derived compounds PEL24-199 and MAGS02-14 (Gay et al., 2018; Tautou et al., 2021).

Results: Curative treatment led to the recovery of short and long-term memory in both animal models. This recovery was associated with a reduced Tau and amyloid pathology together with reduced astrogliosis and neuroinflammation in both models. These effects were obtained with PEL24-199 compounds, which is having only the non-competitive β-secretase activity.

Conclusions: Several drugs having this β-secretase inhibitory activity have been synthesized (Gay et al. 2018) and further investigation is necessary to decipher the mechanism at the crossroad of reducing amyloid and Tau pathology as well as the astrogliosis.
T-TYPE CA2+ CHANNEL ENHANCER SAK3 ACTIVATES THE PROTEASOME ACTIVITIES IN BOTH AD AND DLB MODEL MICE

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Aims: We recently introduced a T-type calcium channel enhancer SAK3 (ethyl-8-methyl-2,4-dioxo-2-(piperidin-1-yl)-2H-spiro[cyclopentane-1,3-imidazo[1,2-a]pyridin]-2-ene-3-carboxylate) for Alzheimer's disease therapeutics. SAK3 restored the decreased proteasome activity in AD mouse brain, thereby promoting the degradation of amyloid plaque and preventing progression of dementia (1). Likewise, the 26S proteasomal activity decreases in the brain of DLB patients. Importantly, SAK3 enhanced the proteasome activity via Ca2+/calmodulin-dependent protein kinase II (CaMKII) activation and Rpt-6 phosphorylation in amyloid precursor protein-knock-in mice. Here, we addressed whether SAK3 promotes the degradation of misfolded α-Syn and the aggregates in α-Syn preformed fibril (PFF)-injected mice.

Methods: Mice were injected with α-Syn PFF in the dorsal striatum, and SAK3 (0.5 or 1.0 mg/kg) was administered orally for 3 months either immediately or during the last month after injection.

Results: SAK3 significantly inhibited the accumulation of fibrillized phosphorylated-α-Syn in the substantia nigra. Consistently, SAK3 significantly recovered mesencephalic dopamine neurons from cell death. Decreased α-Syn accumulation was closely associated with increased proteasome activity. Elevated CaMKII/Rpt-6 signaling possibly mediates the enhanced proteasome activity after SAK3 administration in the cortex and hippocampus. CaMKII/Rpt-6 activation also accounted for improved memory and cognition in α-Syn PFF-injected mice.

Conclusions: These findings strongly suggest that CaMKII/Rpt-6-dependent proteasomal activation by SAK3 ameliorates both AD and DLB pathology and inhibits progression of cognitive impairment in human. SAK3 therapy is ready for first-in-human study because the preclinical studies were completed. This research is partially supported by AMED (20dm0107071). The authors declare no conflict of interests. (1) Izumi H et al., Int J Mol Sci 2020;21:3833.
CNS CLEARANCE OF TOXIC TAU IS PROMOTED BY INHIBITION OF CALCINEURIN IN MICE.

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Aims: Calcineurin (CN) is an important regulator of synaptic function. CN is increased in the CNS of Alzheimer’s Disease (AD) patients and Tg animal models, where CN mediates the detrimental effect of amyloid beta on synapses and memory function. Notably, we reported that humans chronically treated with the CN inhibitor FK506 were protected from developing AD, suggesting a key role of CN in onset and progression of AD. However, the impact of CN on tau, the other amyloid key to later stages of AD progression, remains unresolved. Elucidating the role of CN on tau pathology was the objective of our work.

Methods: Using immunofluorescence microscopy, Western blotting and IP we studied the presence and phosphorylation status of tau, CN, CREB and LC3/2 in the CNS of mice after ICV injection of preformed human tau oligomers (TauO) in the presence of the CN inhibitor FK506. Levels and phosphorylation of tau was also assessed in 3xTgAD mice sub-chronically treated with FK506.

Results: We found that ICV-injected TauO were promptly taken up by neurons and phosphorylated. Inhibition of CN with FK506 very significantly reduced the presence and phosphorylation of exogenously injected TauO, along with evidence of increased autophagy. Notably, the presence of endogenous tau pathology was also significantly reduced in aged 3xTgAD mice after a sub-chronic treatment with FK506.

Conclusions: Our results indicate that inhibition of CN promotes clearance of toxic tau and thus suggest a new target for the future development of an effective, novel treatment for AD. Supported by NIH/NIA R01AG060718 to GT
P2RX7 INHIBITOR SUPPRESSES EXOSOME SECRETION AND DISEASE PHENOTYPE IN P301S TAU TRANSGENIC MICE

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Aims: P2X purinoceptor 7 (P2RX7) is an ATP-gated cation channel, enriched in microglia, able to trigger exosome secretion. Our purpose is to examine the therapeutic effect of an orally applicable, CNS-penetrant P2RX7 specific inhibitor on the early disease stage of a tauopathy mouse model.

Methods: Three-months-old P301S tau mice were treated with P2RX7-specific inhibitor GSK1482160 or vehicle for 30 days, followed by behavioral, biochemical and immunohistochemical assessment. GSK1482160 was also tested for exosome secretion from primary cultured murine astrocytes, neurons and microglia in vitro.

Results: Oral administration of GSK1482160 significantly reduced accumulation of MC1⁺ and Alz50⁺ misfolded tau in hippocampal regions, which was accompanied with reduced neuronal accumulation of Tsg101, an exosome marker. Proximity ligation assay demonstrated complex formation of Alz50⁺ tau and Tsg101 in hippocampal neurons, which was reduced by GSK1482160. On the other hand, GSK1482160 had no effect on microglial ramification or CD68 expression, which was significantly enhanced in P301S mice, or pro/anti-inflammatory cytokine gene expression. Strikingly, GSK1482160-treated P301S mice show significantly improved working and contextual memory. GSK1482160 also significantly increased accumulation of Tsg101 and CD81 in microglia in vivo, suggesting the suppression of P2RX7-induced exosome secretion from microglia. This effect was confirmed in vitro, as ATP-induced secretion of tau-containing exosome was significantly suppressed by GSK1482160 treatment from primary murine microglia, but not from neurons or astrocytes.

Conclusions: Oral administration of P2RX7 inhibitor mitigates disease phenotype in P301S mice, likely by suppressing microglial exosomes’s release. P2RX7 could be a novel therapeutic target during the early stage of tauopathy development.
ON-DEMAND SYMPOSIUM: AD ANIMAL MODELS AND MECHANISTIC & TRANSLATIONAL ASPECTS 3

THERAPEUTIC EFFECTS AND PROTEOMIC ALTERATIONS FOLLOWING MITOCHONDRIAL TRANSFER THERAPY IN 5XFAD TRANSGENIC ALZHEIMER’S DISEASE MICE

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Aims: The pathogenesis of neurodegenerative diseases involves dysfunction of the mitochondria, one of the most important cell organelles in the brain. Aiming to affect as many of the mitochondrial complex functions, rather than a mono-drug related therapy, we investigate the effect of transferring normal intact mitochondria organelles in AD-mice. We have previously shown the beneficial effect of mitochondrial therapy in the short-term AD-mouse model (ICV-injected amyloid-beta), an effect that is mediated via the liver rather than by mitochondria crossing directly the BBB. Here our aim was to investigate the mitochondrial transfer therapy in the chronic 5XFAD transgenic model of AD, for exploring the multiple effects of this therapy, including the involvement of the liver and responsiveness of the brain.

Methods: 5XFAD-mice were IV treated with fresh mitochondria isolated from Hela-cells. Cognitive, histological, biochemical and proteomic analysis were performed.

Results: Amelioration of cognitive deficits, neuronal loss, amyloid-burden and increased brain/liver mitochondrial enzymatic activities was noticed in the mitochondria treated AD-mice. Brain proteomic analysis revealed alterations in various proteins, including phagocytosis-associated, proteasome mediated protein ubiquitination, and synaptic related proteins, - alterations that may be associated with the reduced amyloid plaque burden, with improved cognition, and others. Liver proteomics suggested various metabolic responses, including alterations in the level of Insulin Growth Factor binding protein (IGFbp2), which may affect the availability of the neuroprotective IGF.

Conclusions: Mitochondrial transfer in AD- mice has multiple beneficial effects, involving the responsiveness of various cellular pathways and components in liver and brain. Mitochondrial transfer may offer novel therapeutic approach for AD.
ON-DEMAND SYMPOSIUM: AD ANIMAL MODELS AND MECHANISTIC & TRANSLATIONAL ASPECTS 3

NEURAL STEM CELLS-DERIVED EXOSOMES PREVENT TAU OLIGOMERS-INDUCED SYNAPTIC AND COGNITIVE DYSFUNCTIONS.

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Aims: Synaptic dysfunction induced by toxic oligomers of both Aβ (Aβo) and Tau (TauO) is recognized as one of the earliest key driving events in Alzheimer’s disease (AD) and thus an attractive treatment target. We have previously reported that exosomes released by hippocampal NSC (NSC-exo) render neuronal synapses less vulnerable to Aβo toxicity. In the present work, we aimed to determine whether NSC-exo are also effective against TauO-induced synaptic and memory deficits.

Methods: Adult male C57/Blk6 mice received intracerebroventricular (icv) injections of exosomes, isolated from the culture media of hippocampal NSC (NSC-exo) or mature neurons (MN-exo), or PBS. For electrophysiological assessment of synaptic function, mice were euthanized 24 hours later, and brain slices were incubated with TauO (50 nM) for 1 hr prior to high-frequency-induced long-term potentiation (LTP) recording. For behavioral assessments of memory function, TauO were delivered icv 24 hours after exosomes injections, and the mice were subjected to the novel object recognition (NOR) test 4 hours later.

Results: We found that TauO-induced suppression of LTP expression in the hippocampus was prevented by icv delivery of NSC-exo but not by MN-exo or PBS. We further found that NSC-exo prevented TauO-associated memory impairments in the NOR test, while MN-exo were ineffective.

Conclusions: Taken together, these results, and our previously published work, show that NSC-exo provide significant protection against synaptic dysfunction and associated memory deficits induced by both AβO and TauO and may therefore provide an unprecedented therapeutic strategy to prevent or delay synaptic damage in the AD brain.
TARGETING MICROGLIAL MIR-155 ENHANCES MICROGLIA RESPONSE TO NEURODEGENERATION AND IMPROVES COGNITIVE FUNCTIONS IN ALZHEIMER’S DISEASE MODEL

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Aims: Microglia are the resident immune cells in the CNS that regulate brain development, maintenance of the neuronal network, and neurodegenerative disease progression. Our group identified homeostatic (M0) and neurodegenerative microglia (M GnD), also referred to as disease-associated microglia (DAM), that are regulated by the reciprocal suppression of TGFβ and induction of APOE signaling in multiple neurodegenerative disease models. Understanding whether the M GnD phenotype is beneficial or detrimental in Alzheimer’s disease (AD) progression is currently one of the major questions for therapeutic approaches in AD. miR-155 is a pro-inflammatory miRNA that modulates the inflammatory responses in innate immunity. However, its role in AD pathogenesis remains unknown.

Methods: We utilized multiple approaches including Smartseq2 and single-cell RNAseq, proteomics, immunohistochemistry, and behavioral tests.

Results: We found that conditional ablation of microglial miR-155 at 1.5 months of age in APP/PS1 mice significantly increased the expression of M GnD genes, including Apoe, Clec7a, and Spp1, enhanced interferon signaling, and suppressed inflammatory signaling, including TNF-α and NF-κB in 4-month-old APP/PS1 mice. Moreover, using immunohistochemistry and proteomics, we found the induction of the M GnD microglial phenotype was correlated with increased amyloid plaque compaction, reduced neuritic dystrophy, and enhanced microglial phagocytosis and synaptogenesis. Conditional ablation of microglial miR-155 in APP/PS1 mice at 1.5 months of age improved behavioral cognitive performance based on Y maze and fear conditioning at 8 months of age.

Conclusions: These findings support the beneficial role of M GnD microglia in chronic neurodegeneration and serve as the basis for the therapeutic strategy to induce M GnD microglia for the treatment of AD.
ON-DEMAND SYMPOSIUM: AD ANIMAL MODELS AND MECHANISTIC & TRANSLATIONAL ASPECTS 3

DISCOVERY OF A NOVEL THERAPEUTIC ANTIBODY (ABL303) TARGETING TREM2 FOR ALZHEIMER’S DISEASE

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Aims: Variants of triggering receptor expressed on myeloid cells 2 (TREM2) increase the risk of sporadic Alzheimer’s disease (AD). TREM2 deficiency facilitates microglial accumulation around Aβ plaques, thus leading to Aβ plaque formation and injury of adjacent neurons. Therefore, TREM2 is emerging as a valid therapeutic target for AD. Here we show that lead candidates of ABL303, agonistic anti-TREM2 antibodies promote microglial activation and recruitment of microglia around amyloid plaques, and reduces amyloid deposition.

Methods: The agonistic activity of ABL303 candidates were evaluated by 2 different assays in vitro first: 1) Induction of spleen tyrosine kinase phosphorylation (p-Syk) in HEK293 cells expressing a chimeric receptor of extracellular domain of TREM2 and its signaling adapter DAP12, 2) increase in survival of human perinuclear blood mononuclear cell (PBMC)-derived macrophages. ABL303 candidates’ in vivo functions in microglial activation and phagocytosis was assessed by their direct intracranial injection into hippocampus of 5xFAD mice.

Results: ABL303 candidates clearly induced the p-Syk in the HEK293 cells expressing the chimeric receptor. ABL303 candidates increased the survival of human macrophages in the absence of M-CSF. Both assays showed clear dose response. Intracranial injection of the ABL303 candidates resulted in recruitment and activation of microglia surrounding amyloid plaques and reduced plaque burden in 5xFAD mice.

Conclusions: ABL303 candidates exerted agonistic activation of TREM2 receptor signaling and enhancement of TREM2-dependent microglial activity, leading to reduction of amyloid burden in AD model. This suggests that ABL303 candidates may function as novel, effective antibody therapeutics to alleviate AD pathology.
ON-DEMAND SYMPOSIUM: AD ANIMAL MODELS AND MECHANISTIC & TRANSLATIONAL ASPECTS 3

MULTI-OMICS TARGETOME PROFILING OF MICRORNA-132 FOR THERAPEUTIC TARGETING IN AD

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Aims: microRNA profiles in the brain of Alzheimer’s disease (AD) patients are altered early on along disease progression, suggesting that microRNAs may contribute to AD pathology. Of particular interest is microRNA-132 (miR-132), which is consistently and robustly downregulated in AD patient brain and has been shown to be involved in pivotal processes in the central nervous system that are also affected in AD. Targeting microRNAs could represent a promising multi-pathway therapeutic strategy in multigenic disorders, like AD. In order to systematically assess the therapeutic potential of miR-132, its targetome and the pertinent disease-relevant or toxicity-associated molecular pathways need to be systematically profiled. Taken the cellular complexity of brain tissue and its impact on AD pathology, single-cell genome-wide approaches are required to achieve high-resolution molecular mapping.

Methods: Intracerebroventricular infusion of miR-132 synthetic mimics or antisense oligonucleotides was employed to elevate or knock down miR-132 levels in mouse brain. Hippocampal tissue was subsequently processed and subjected to complementary unbiased proteomics analysis (MaxLFQ) and single-cell RNA sequencing (10X Genomics) to identify direct miR-132 targets and miR-132-regulated molecular pathways.

Results: Following integrative analysis of the resulting datasets, miR-132 shows to regulate distinct molecular pathways in different cell types in the brain. In addition, in vivo modulation of miR-132 could potentially affect the proportion of cells present in disease-associated cellular states, suggesting a regulatory role in cell fate or activation decisions.

Conclusions: Overall, our data suggest that miR-132 represents a potent regulator of previously reported and novel molecular pathways with multilayered implications in AD pathology.
BRAIN PHARMACOKINETICS OF AMYLOID-BETA TARGETING BISPECIFIC ANTIBODY IN ALZHEIMER’S DISEASE MOUSE MODEL

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Aims: Amyloid-β (Aβ) targeting antibodies are a promising way to decrease Alzheimer’s disease (AD) brain pathology and possibly even slow down or stop the progression of the disease. One of the main challenges in immunotherapies is low access of antibodies into the brain, but bispecific antibodies can access to the brain better than traditional antibodies by using the transferrin receptor (TfR) as a shuttle for transport through the blood-brain barrier. However, there is only limited information of antibody brain pharmacokinetics available. The aim of the study was to compare brain pharmacokinetics of Aβ targeting monospecific and bispecific antibodies in mouse models of AD.

Methods: Cerebral microdialysis was used to measure intravenously injected monospecific Aβ targeting antibody 3D6 or bispecific Aβ-TfR targeting 3D6-8D3 antibody in the interstitial fluid of hippocampus in wild-type and AD modeling knock-in mice (AppNLGF) expressing mutated human Aβ precursor protein (APP). Distribution of antibodies in the different areas of the brain and in the peripheral tissue were studied by ex vivo autoradiography and biodistribution.

Results: Concentration of bispecific antibody was elevated in the interstitial fluid and in the brain tissue compared to monospecific antibody 4-6 hours post-injection showing higher brain penetration of bispecific antibody compared to monospecific antibody.

Conclusions: Improving brain access by using bispecific antibodies could provide significant improvement to current immunotherapies. Better understanding of antibody pharmacokinetics in the brain is an essential step to develop safe, effective and reasonably priced antibody treatments for AD.
ON-DEMAND SYMPOSIUM: AD ANIMAL MODELS AND MECHANISTIC & TRANSLATIONAL ASPECTS 3

TREATMENT WITH A SMALL MOLECULE INHIBITOR OF TAU SELF-ASSOCIATION REDUCED TAU AGGREGATION IN TWO MOUSE MODELS OF TAUOPATHY USING BOTH PREVENTIVE AND THERAPEUTIC STUDY DESIGNS

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Aims: The overall aim of the preventive and therapeutic studies in the htau and JNPL3 mice was to test the hypothesis that targeting tau self-association in vivo could prevent tau aggregation in young mice and inhibit the progressive accumulation of tau aggregates in aged mice and improve their behavior.

Methods: Studies were independently performed with blinded lots of feed for the vehicle and treatment groups. htau and JNPL3 mice were treated from 3 to 7 months of age in preventive studies and aged to seven months and treated from 7 to 12 months in therapeutic studies. Biochemical and immunocytochemistry methods are detailed in publications (Forest SK, et al., J Alzheimers Dis. 2013, 33:463-71; Davidowitz EJ et al., J Alzheimers Dis. 2020, 73:147-161).

Results: Preventive studies in htau and JNPL3 mice showed that treatment with OLX-07010 reduced the levels of self-associated tau and phosphorylated insoluble tau aggregates. In the JNPL3 therapeutic study, treatment groups at 12 months-of-age had levels of self-associated tau, Sarkosyl insoluble tau and heat stable tau in the cortex at or below levels in the baseline group at 7 months-of-age. Reduction in tau pathology in treatment groups correlated with their improved motor performance.

Conclusions: Treatment with OLX-07010 was effective in preventing the accumulation of tau aggregates in young mice and in inhibiting the progression of tau aggregation in aged mice in models representing tau aggregation in 4R tauopathies and Alzheimer’s disease showing the broad potential application of this treatment for Alzheimer’s disease and rare tauopathies supporting its preclinical development for clinical studies.
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Aims: There are several extra-neuronal complications observed in Alzheimer’s disease (AD) patients. Falls and fractures are predominant in AD and management of bone health during AD is crucial to ameliorate the severity of the disease as well as prevent mortality. This study is focused on identifying alterations in the bone structure and function in different preclinical models of AD.

Methods: Four distinct preclinical models of AD: MAPT P301S Tg+, 5xFAD, FDD and PSEN mutations were investigated for their bone microarchitecture at 10-12 months of age (10 males and females per group) and compared to age-matched wild type mice. Briefly, the left femurs were assessed by using microCT for cortical and trabecular properties and further subjected to biomechanical testing.

Results: There was reduction in bone mineral density at one month in PSEN KI mice, even before the onset of amyloid pathology which happens at six months of age in these mice. The reduction in the cortical and trabecular parameters in the PSEN KI mice compared to their age-matched wild type controls is seen as early as four months in females only and is incremental with age. There is no change in the bone mineral density MAPT P301S Tg+ mice, but significant reductions in cortical, trabecular properties and mechanical strength in male MAPT P301S Tg+ mice. FDDTg+ mice has reduced cortical and trabecular properties in males and females.

Conclusions: Several preclinical models of AD have shown distinct changes in the bone phenotype with sex-dependent effects on the structure, mechanical and composition of the bone.
ON-DEMAND SYMPOSIUM: DIAGNOSTIC ACCURACY, BIOMARKERS IN AD, CAA, PD, LBD

THE DETECTION OF SALIVARY PHOSPHORYLATED TAU IS NOT ASSOCIATED TO ALZHEIMER’S DISEASE, CEREBROSPINAL FLUID OR BLOOD BIOMARKERS

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Aims: Phosphorylated tau (p-tau) in plasma and cerebrospinal fluid (CSF) have great value to monitor neurodegeneration in Alzheimer’s Disease (AD). Salivary biomarkers are non-invasive and inexpensive, and in this study, our aim was to investigate the use of salivary p-tau to diagnose AD.

Methods: Saliva and plasma p-tau were measured in 119 participants in a mixed memory clinic population by a modified single molecule array (Simoa) method. Salivary p-tau181 was analysed in ratio with total protein. Mass spectrometry with immunoprecipitation (IPMS) was used to explore salivary p-tau181, and immunohistochemistry (IHC) determined the presence of p-tau species in salivary gland tissue.

Results: Participants were classified as cognitive unimpaired (CU) or impaired (CI). Amyloid-β pathology was determined in the CI group (Aβ− / Aβ+). Salivary p-tau181 levels did not change between CU, CI Aβ− and CI Aβ+, although plasma p-tau181 was significantly elevated in CI Aβ+, compared to CU and CI Aβ− (P< 0.001). No correlation existed between CSF and saliva p-tau181. However, in CI Aβ+ patients a weak positive correlation was observed (r = 0.228, P<0.05). IPMS and IHC confirmed the presence of p-tau species in salivary fluid and gland tissues, respectively. The latter illustrating a differential expression between p-tau epitopes, p-tau181, p-tau217 and p-tau231.

Conclusions: Our data confirms the detection and expression of multiple p-tau forms in salivary fluid and gland tissue. While, at the group level, there is no change in salivary p-tau levels, a weak but significant relationship does exist with CSF p-tau181 in the CI Aβ+ group.
SKIN BIOMARKERS FOR EARLY DIAGNOSIS OF AD AND ADRD

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Aims: The current diagnosis of AD often requires the detection of β-amyloid and/or tau inclusions in the brain. To identify biomarkers in readily accessible peripheral tissues, we have utilized the real-time quaking-induced conversion (RT-QuIC) to detect misfolded tau aggregates in the skin.

Methods: We have developed a robust and ultrasensitive RT-QuIC assay to detect tau aggregates in the skin of patients with AD and AD-related dementia (ADRD).

Results: We first validated our tau RT-QuIC assay using brain homogenates (BH) of AD and non-tauopathy (NT) controls. We found that our RT-QuIC assay readily detected the seeding activity of 3-repeat (3R)- and 4R-tau isoforms in BH of AD but not in NT controls. We then examined skin samples from neuropathologically confirmed AD cases. The RT-QuIC reactions seeded by the skin samples from AD exhibited markedly enhanced responses as compared to the NT controls. Moreover, the skin tau RT-QuIC assay achieved high sensitivity and specificity for the diagnosis of AD. We also performed α-synuclein RT-QuIC assay on the skin samples of AD and dementia with Lewy bodies (DLB), facilitating differential diagnosis of pure AD and DLB from that with mixed AD/DLB pathologies. Taken together, we have developed a novel RT-QuIC assay for the detection of tau-seeding activity in the skin to serve as a peripheral biomarker for the diagnosis of AD.

Conclusions: Our study have demonstrated that skin tau aggregates can be readily detected by the ultrasensitive RT-QuIC assay, and may serve as a novel skin biomarker for accurate and early diagnoses of AD and ADRD.
Aims: The aim of this work is to identify, quantify and validate microRNAs (miRNAs) in plasma samples as potential biomarkers for AD.

Methods: Epigenomic analysis was carried out in plasma samples from 46 participants (8 preclinical AD, 19 mild cognitive impairment (MCI) due to AD and 19 healthy participants). MiRNAs sequencing was carried out by NGS NextSeq 550 platform. Then, miRNA differential expression analysis comparing different methods (DESeq2, edgeR, NOISeq) were carried out. Differentially expressed miRNAs between groups were selected and targeted quantification was carried out by RT-PCR in order to validate the results.

Results: From differential expression studies, 11 miRNAs were selected. They showed a number of acceptable counts in the samples. In the PCR analysis, some of the miRNAs showed values under the limit of quantification. None of the miRNAs analyzed showed significant differences between the groups. In general, between MCI-AD and healthy groups there was not observed any tendency. However, some of the miRNAs showed increased (miR486-5p, miR-486-3p, miR-320b) or decreased (miR-29a-3p) in preclinical AD compared to healthy participants, while no differences were found between MCI-AD and healthy participants.

Conclusions: Different miRNAs showed slightly increased or decreased levels in patients with preclinical AD compared to healthy participants. However, quantitative analysis in an external cohort should be carried out in order to validate these results.
AN ELISA FOR VAMP2, A CSF MARKER FOR SYNAPTIC INTEGRITY IN AD AND OTHER NEUROLOGICAL DISEASES

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Aims: A target MS approach demonstrated the possible value of vesicle-associated membrane protein 2 (VAMP2) as a marker for synaptic integrity. A Simoa based assay for VAMP2 confirmed its value as a biomarker for AD, but an ELISA would accelerate future clinical evaluations.

Methods: Synthetic peptides were used to generate high-affine VAMP2 rabbit monoclonals. New immuno-assays formats were evaluated for analytical sensitivity and optimized for ELISA quantification.

Results: Rabbit monoclonal G11 as coating antibody and mouse monoclonal 15E4 as detector was tested for VAMP2-specificity on all three recombinant VAMP proteins. Specificity of this monoclonal antibody combination was 6 times higher for VAMP2 than for VAMP1, while no cross-reactivity for VAMP3 was found. A partial analytical validation of the assay was carried out. A clinical study in the Alzheimer continuum demonstrated normal concentrations in control samples and pre-clinical stage 1. In pre-clinical stage 2, prodromal AD, and AD dementia, elevated VAMP2 concentrations were measured. These results correlated highly with our previous results on Simoa.

Conclusions: We were able to build a VAMP2 ELISA that performs as well as our Simoa assay and confirms that VAMP2 is a marker for synaptic integrity in the AD continuum.
ON-DEMAND SYMPOSIUM: DIAGNOSTIC ACCURACY, BIOMARKERS IN AD, CAA, PD, LBD

MATRIX METALLOPROTEASES AND THEIR TISSUE INHIBITORS IN CEREBROSPINAL FLUID AS POTENTIAL BIOMARKERS FOR SPORADIC CEREBRAL AMYLOID ANGIOPATHY

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Aims: Sporadic cerebral amyloid angiopathy (sCAA) is characterized by the deposition of amyloid beta peptides in the cerebral vasculature and the subsequent progressive degradation of cerebral vessels, predisposing cerebral bleeding. An aggravated proteolytic effect of matrix metalloproteases (MMPs) is theorized to contribute to this phenomenon, which would cause destabilization of the integrity of the vascular wall. This potential disruption in the balance between activity of MMPs and their tissue-type inhibitors (TIMPs) could be reflected in the cerebrospinal fluid of sCAA patients, which makes them interesting targets for biomarker research.

Methods: We analyzed levels of MMPs-2, -9 and -14, as well as the levels of TIMP-1, -2, and -3 in the CSF of sCAA patients in varying group sizes using ELISA (see Figure 1). Samples were collected from the Radboud University Medical Center, Leiden University Medical Center and Massachusetts General Hospital.

Results: Univariate analyses resulted in significant elevations of MMP-9 and TIMP-3 levels in sCAA patients versus controls (p = 0.026 and 0.027, respectively). Adjusting for age of subjects using linear regression, significant elevations of TIMP-3 levels were retained (p = 0.021). Additionally, the ratios of MMP-2/TIMP-2, MMP-14/TIMP-2 and MMP-14/TIMP-3 levels in CSF proved to be (highly) effective in discriminating sCAA patients from controls (p = 0.019, p = 0.044 and p=0.001 respectively).

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>N (pts(ctrls))</th>
<th>sCAA (IQR)</th>
<th>Controls (IQR)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-2 (ng/mL)</td>
<td>36/40</td>
<td>29.9 (25.9 – 37.9)</td>
<td>28.9 (24.5 – 32.8)</td>
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<tr>
<td>MMP-9 (ng/mL)</td>
<td>27/40</td>
<td>0.6 (0.4 – 1.1)</td>
<td>0.9 (0.7 – 2.3)</td>
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<tr>
<td>MMP-14 (ng/mL)</td>
<td>43/40</td>
<td>3.0 (2.2 – 3.8)</td>
<td>2.4 (2.1 – 3.2)</td>
<td>0.147</td>
</tr>
<tr>
<td>TIMP-1 (ng/mL)</td>
<td>43/36</td>
<td>40.3 (31.2 – 57.7)</td>
<td>41.5 (3.6 – 5.8)</td>
<td>0.798</td>
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<tr>
<td>TIMP-2 (ng/mL)</td>
<td>43/39</td>
<td>48.3 (42.8 – 53.0)</td>
<td>51.0 (4.4 – 5.8)</td>
<td>0.186</td>
</tr>
<tr>
<td>TIMP-3 (pg/mL)</td>
<td>37/37</td>
<td>88.8 (54.7 – 120.7)</td>
<td>123.2 (71.1 – 192.8)</td>
<td>0.027 (*)</td>
</tr>
</tbody>
</table>

Conclusions: Levels of several MMPs and TIMPs were shown to be different in sCAA compared to controls. This indicates disruption of the delicate balance between MMPs and TIMPs in sCAA, which could provide interesting insight into sCAA pathophysiology and future biomarker research.
A NOVEL IMMUNOASSAY TO MEASURE DOPA-DECARBOXYLASE IN CSF FOR THE DIFFERENTIAL DIAGNOSIS OF DEMENTIA WITH LEWY BODIES

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Aims: Dementia with Lewy bodies (DLB) is one of the most common dementia forms, but is frequently misdiagnosed. A DLB-specific biofluid biomarker could help to differentiate DLB from other dementias. In a previous proteomics study, we identified DOPA-Decarboxylase (DDC) as a potential cerebrospinal fluid (CSF) biomarker candidate for DLB. DDC is dysregulated in Parkinson’s Disease, and α-synuclein has been found to inhibit DDC activity, indicating a possible involvement of DDC in the pathology of α-synucleinopathies. Our aim was to develop and validate an immunoassay specific for the detection of DDC in CSF.

Methods: We developed a novel sandwich immunoassay to measure DDC protein levels in CSF on the highly sensitive Ella-platform. This assay was technically validated for the parameters sensitivity, precision, linearity, and parallelism; the acceptable range was set to <20% or 85%-115% from the reference.

Results: We determined a lower limit of quantification of 5.24 pg/mL. The mean intra- and inter-assay coefficient of variation were 8.6% and 21.5%, respectively. Parallelism (87%-110%) fell within the acceptable range, indicating the same binding properties of the antibodies towards the calibrator and the endogenous protein, and no effect of the matrix. Dilution linearity (91%-108%) was in the acceptable range, and no hook-effect was observed.

Conclusions: We successfully developed and technically validated a novel immunoassay for the measurement of DDC levels in CSF. Next, we will proceed with clinical validation to evaluate the potential use of DDC as a biomarker to differentiate DLB from other dementia types.
DIFFERENT MARKERS TO DEFINE SUSPECTED NON-ALZHEIMER’S DISEASE PATHOPHYSIOLOGY IN INDIVIDUALS WITH MILD COGNITIVE IMPAIRMENT SHOW DISTINCT CSF PROTEOMIC PROFILES

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Aims: Suspected non-Alzheimer’s disease pathophysiology (SNAP) is a biomarker concept describing individuals with normal amyloid-β (Aβ) but abnormal neurodegeneration biomarkers. We investigated the pathophysiology of SNAP in individuals with mild cognitive impairment (MCI), defined using different neurodegeneration markers, i.e. total tau (t-tau), neurofilament light (NfL) and hippocampal volume (HCV).

Methods: We included 198 individuals from the European EMIF-AD MBD and Maastricht BB-ACL study. Using CSF Aβ1-42 (A) and t-tau, NfL or HCV (N), individuals were classified as cognitively normal (CN) A-N-, MCI-SNAP A-N+, and MCI A+N+. CSF proteomic data were generated using TMT mass spectrometry and compared between groups using ANOVA adjusted for age, sex and APOE-ε4. Gene Ontology analyses were performed.

Results: Compared to CN, SNAP-Tau showed decreased concentrations of 72 proteins related to hemostasis and oxidative stress, and increased levels of 78 proteins related to nervous system. Compared to A+Tau+, SNAP-Tau showed decreased concentrations of 133 proteins related to extracellular matrix, lipids, hemostasis and oxidative stress (Figure 1). Compared to CN, SNAP-NfL showed 74 decreased proteins related to lipids, oxidative stress and hemostasis. Compared to A+NfL+, SNAP-NfL showed decreased concentrations of 120 proteins related to energy metabolism and oxidative stress (Figure 2). Compared to CN, SNAP-HCV showed decreased levels of 197 proteins related to nervous system and angiogenesis. Compared to A+HCV+, SNAP-HCV presented decreased concentrations of 149 proteins related to energy metabolism and nervous system (Figure 3).
Figure 1. Proteomic comparison between CN A−T-tau− (n=63), MCI-SNAP A−T-tau+ (n=21) and MCI A+T-tau+ (n=71). (A) Venn diagram representing the increased/decreased proteins for each comparison. (B) Heatmap representing the log2 fold-change values between groups for the 425 dysregulated proteins in at least one of the comparisons. (C-F) Selected Gene Ontology biological process terms for the decreased and increased proteins in MCI-SNAP A−T-tau+ compared to CN A−T-tau−. For the decreased proteins in MCI-SNAP A+T-tau− compared to CN A−T-tau− and for the decreased proteins in MCI-SNAP A−T-tau− compared to both CN A−T-tau− and MCI A+T-tau+. Hemostasis-related pathways are red, oxidative stress pathways are grey, proteins-linked pathways are purple, immune system-related proteins are pink, pathways related to nervous system are blue, extracellular matrix pathways are green, lipids-linked pathways are yellow and pathways linked with energy metabolism are orange.
Figure 2. Proteomic comparison between CN A−/NIL− (n=48), MCI−SNAP A−/NIL− (n=34) and MCI A+/NIL− (n=57). (A) Venn diagram representing the increased/decreased proteins for each comparison. (B) Heatmap representing the log2 fold-change values between groups for the 224 dysregulated proteins in at least one of the comparisons. (C-F) Selected Gene Ontology biological process terms for the decreased proteins in MCI−SNAP A−/NIL− compared to CN A−/NIL−, for the decreased proteins in MCI−SNAP A−/NIL− compared to MCI A+/NIL−, and for the decreased proteins in MCI−SNAP A−/NIL− compared to both CN A−/NIL− and MCI A+/NIL−. Lipid-related pathways are yellow, oxidative stress pathways are grey, hemostasis-related pathways are red, proteins-linked pathways are purple, pathways linked with energy metabolism are orange and pathways related to nervous system are blue.
Conclusions: MCI-SNAP showed decreased protein levels compared to CN and MCI A+N+. T-tau, NFL and HCV in MCI-SNAP represent distinct conditions with different proteomic profiles.
LONGITUDINAL CHANGES IN BLOOD-BASED BIOMARKERS IN CHRONIC MODERATE TO SEVERE TRAUMATIC BRAIN INJURY

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Aims: Little is known about the changes in blood-based biomarkers and their relation to outcome, years after Traumatic Brain Injury. This prospective study aims to gather preliminary data on changes in blood-based biomarkers across time and to relate these changes to changes in outcome measures as well as to changes in cerebral structure and neurophysiologic activity.

Methods: Eight patients with moderate-to-severe TBI were recruited (7 males, 35 ± 7.6 years old, 5 severe TBI, 17.52 ± 3.84 months post-injury). The following data were evaluated at monthly intervals across 6 time-points: a) Blood-based biomarkers, including GFAP, NSE, S100A12, SDBP145, UCH-L1, T-tau, P-tau, and P-tau/T-tau ratio; b) Magnetic Resonance Imaging (MRI) to evaluate changes in brain structure using FMRIB Software Library (FSL); c) Resting state electroencephalograms (EEG) to evaluate changes in brain function using EEGLAB; and d) Outcome measures to assess cognition, emotion and functional recovery (i.e., MOCA, RBANS, BDI-II and DRS).

Results: Changes in P-tau levels were found across time [p=0.007]. P-tau was positively related to functional [p<0.001] and cognitive [p=0.006] outcomes, and negatively related to the severity of depression, 6 months later [R=-0.901; p=.006]. P-tau and P-tau/T-tau ratio were also positively correlated to shape change in subcortical areas such as brainstem [T(7)=4.71, p=0.008] and putamen [T(7)=3.25, p = 0.012].

Conclusions: Our study provides preliminary findings that suggest a relationship between P-tau and the recovery of patients with chronic TBI. Further investigation in a larger cohort is warranted to validate this biomarker and better understand the mechanism of neural recovery in chronic patients.
Aims: To use electron cryo-microscopy (cryo-EM) for determining the structures of tau filament from a number of tauopathies. This will lead to a better understanding of disease pathogenesis and inform ongoing efforts to develop more specific and sensitive tau biomarkers.

Methods: We determined the structures of tau filaments from the brains of individuals with progressive supranuclear palsy (PSP), globular glial tauopathy (GGT), argyrophilic grain disease (AGD), aging-related tau astrogliopathy (ARTAG), familial British dementia (FBD), familial Danish dementia (FDD), mutations at positions +3 or +16 in intron 10 of MAPT (the microtubule-associated protein tau gene).

Results: The structures of tau filaments from PSP define a novel three-layered fold. Moreover, the tau filament structures from GGT are similar to those from PSP. Distinct tau filament structures that are intermediate between those of GGT and PSP are shown in a case diagnosed as PSP. The tau filament fold of AGD differs from the above and resembles the four-layered corticobasal degeneration (CBD) fold. The AGD fold is also observed in ARTAG. Tau protofilament structures from inherited cases with mutations +3 or +16 in intron 10 of MAPT are also identical to those from AGD. Finally, tau filament structures from cases of FBD and FDD are the same as those from Alzheimer’s disease and primary age-related tauopathy (PART).

Conclusions: These findings suggest a hierarchical classification of tauopathies on the basis of their filament folds, which complements clinical diagnosis and neuropathology and allows the identification of new entities.
ON-DEMAND SYMPOSIUM: MOLECULAR MECHANISMS IN PROTEINOPATHY, TAUOPATHIES AND SYNUCLEINOPATHIES

ESTIMATION AND VALIDATION OF 18F-FLORTAUCIPIR DATA-DRIVEN CUT-OFF VALUES FOR TAU STAGING IN ALZHEIMER’S DISEASE.

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Aims: The identification of accurate cut-offs for tau PET positivity is of paramount importance for Alzheimer’s disease (AD) diagnosis. Here, we used a data-driven approach to determine cut-offs for in-vivo tau staging in AD.

Methods: Data were derived from ADNI and the Geneva Memory Center (GMC) cohorts. Amyloid negative (Aβ-) cognitively normal (CN) and amyloid positive (Aβ+) CN, mild cognitive impairment (MCI), and AD subjects were included. 18F-Flortaucipir SUVRs were calculated for the temporal meta-ROI and for the Braak-based stages I-VI. SUVR cut-offs were estimated applying the Gaussian mixture model on Aβ- CN and Aβ+ AD (ADNI; n=269). Potential confounding factors (age, sex, APOE e4) were also included as covariates to assess their effect on cut-offs estimation. Sensitivity and classification analyses were conducted on both cohorts.

Results: Cut-offs were 1.34 for temporal meta-ROI, and ranging from 1.22 (VI) to 1.36 (IV) for Braak-based stages. No effect of the confounds was reported. In the ADNI cohort, the sensitivity increased from Aβ- CN to Aβ+ CN, MCI, and AD for the temporal meta-ROI (3%, 12%, 45 %, 85%) and stages: I-II, 5%, 18%, 57%, 91%; III, 2%, 9%, 44%, 88%; IV, 2%, 10%, 36%, 82%; V, 6%, 5%, 12%, 12%; VI, 1% (CNs), 8%, 9%. The majority of CNs was tau negative (>74%), MCI were either tau negative or stage IV (>30%), and AD were predominantly stage IV (>70%). Similar patterns were reported for the GMC cohort.

Conclusions: Data-driven 18F- Flortaucipir SUVR cut-offs could be useful for in-vivo tau staging in AD.
ON-DEMAND SYMPOSIUM: MOLECULAR MECHANISMS IN PROTEINOPATHY, TAUOPATHIES AND SYNUCLEINOPATHIES

LOSS OF TAU EXPRESSION ATTENUATES NEURODEGENERATION ASSOCIATED ALPHA-SYNUCLEIN PATHOLOGY IN VIVO

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Aims: Neuronal dysfunction and degeneration linked to a-synuclein (aS) pathology is thought to be responsible for the neurodegeneration in Parkinson's Disease (PD) and related Lewy Body Dementia (LBD). Studies indicate bidirectional pathological relationships between aS pathology and tau abnormalities. For example, we showed that A53T mutant human aS (HuaS) can cause tau dependent post-synaptic and cognitive deficits. Therefore, we examined whether tau is involved in the onset and progression of overt a-synucleinopathy.

Methods: We induced a-synucleinopathy by intramuscular (IM) injections of HuaS preformed fibrils (PFF) in the A53T mutant transgenic mice (TgA53T), in mTau−/− or wildtype background.

Results: IM inoculation of TgA53T mice leads to motor dysfunction onset by ~70 days post inoculation (dpi) and end-stage paralysis by ~100 dpi. Significantly, TgA53T/mTau−/− mice exhibit reduced motor deficits at 70 dpi and delayed onset of paralysis. Analysis of neuropathology shows that end-stage TgA53T mice and TgA53T/mTau−/− mice show comparable pathology. However, at 70 dpi, TgA53T/mTau−/− mice had modest yet significant reductions of a-synucleinopathy, including the loss of ventral motor neurons. Similarly, in vitro application of PFF to primary hippocampal neurons demonstrated no change of PFF induced pS129aS aggregation as a function of tau expression, but neurotoxic indicators including morphology and postsynaptic density deficits were prevented with tau removal.

Conclusions: While tau expression does not impact onset and progression of aS aggregation, loss of tau expression protects neurons from downstream toxic effects of aS aggregation. Therefore, tau reduction and the pathways activated may represent novel therapeutic targets for a-synucleinopathy.
ON-DEMAND SYMPOSIUM: MOLECULAR MECHANISMS IN PROTEINOPATHY, TAUOPATHIES AND SYNUCLEINOPATHIES

PROTEOMIC ANALYSIS OF THE PARKINSON DISEASE HETEROGENEITY: A STUDY ON PPMI PATIENT COHORTS


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Aims: Proteomic and genomic analyses of biological matrices such as cerebrospinal fluid (CSF) open opportunities to scrutinize Parkinson’s Disease (PD) heterogeneity. We examined three cohorts from the Parkinson Progression Marker Initiative (PPMI): manifest PD patients carrying LRRK2 or GBA mutations and idiopathic patients, each with their corresponding controls.

Methods: This is to date the most comprehensive proteomics profiling in CSF of PD patients (1113 samples, 4785 markers).

Results: We identified significant differences between patients and controls within the cohorts: 6 proteins differed among GBA mutation carriers, 7 proteins among LRRK2 mutation carriers and 23 proteins for the idiopathic cohort. Many of these proteins are known in PD, but we also highlighted new markers that may help to expand our knowledge around the disease. We also performed a stratification of idiopathic patients based on the proteomic profile. A combination of network analysis and consensus clustering revealed two patient classes. Differences between these classes fit a proposal of two separate mechanisms for neurodegeneration in PD, one mediated by CD4+ T-cells, the other by microglia and activation of cytotoxic CD8+ T-cells. Additionally, we identified 62 proteins causally associated with PD using two sample mendelian randomization, out of which 3 have a strong co-localization signal.

Conclusions: Overall, our results showed molecular heterogeneity in the different cohorts. The proteomic biomarkers, the unique signature of the CSF as well as the causal targets we identified using both proteomics and genetics may influence how we define PD and the strategies to approach the identification of therapeutic targets.
FINE PARTICULATE MATTER IN AIR POLLUTANTS PROMOTES NEUROINFLAMMATION AND EXACERBATES TAUOPATHY IN HUMAN MINIBRAIN

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Aims: Fine particulate matter 2.5 (PM2.5), a major toxic component among air pollutants, highlights as a global health concern that may induce neurodegeneration in Alzheimer’s disease (AD) brains. However, effects of PM2.5-induced tauopathy in AD brains have not been clarified yet. Here, we aim to investigate how the crosstalk of neurons and glia in the air pollutant-exposed brains promotes tau accumulation that may contribute to AD pathogenesis.

Methods: We engineered PM2.5-polluted human Mini-Brains (PMBs) in a microscale reconstituting key aspects of human central immunity under the PM2.5 exposure. First, we confirmed the penetration of PM2.5 across in vitro blood-brain barrier (BBB) on the Transwell plates. Next, we utilized our PMBs to interpret the crosstalk among microglia, astrocytes, as well as neurons and unpuzzle the underlying mechanisms of PM-driven tauopathy layer-by-layer.

Results: Our results showed that the BBB-penetrating PM2.5 initiated reactive astrogliosis, producing oxidative stress (H2O2) and resulting in slight reduction in neuron viability. In addition, the reactive astrocytes produced chemoattractants (CCL1 and CCL2), which induced microglial infiltration. Interestingly, the infiltrated microglia further obtained M1 phenotype induced by interleukin-1β and interferon-γ from the reactive astrocytes under the PM2.5 exposure. Moreover, the M1 microglia exerted the significant accumulation of tau proteins in the PMB models presumably by microglia-driven interleukin-6. Lastly, we observed that M1 microglia further promoted synaptic impairment and neuronal death in advance by nitric oxide.

Conclusions: Our study revealed that PM2.5-driven neuroinflammation could be a potential risk factor for AD by promoting tauopathy and neurodegeneration.
Aims: Pathogenic Tau mutations interfere with the binding of Tau to microtubuli resulting in the relocation of Tau to different neuronal compartments. We showed that Tau interacts with synaptic vesicles and that excessive Tau levels at presynapses results in the sequestration of synaptic vesicles compromising presynaptic function. Our aim is to understand the molecular mechanisms resulting in the accumulation of Tau at presynapses.

Methods: We generated several new transgenic fly lines expressing human pathogenic Tau to unravel pathogenic mechanisms resulting in presynaptic Tau pathology and increased endosomal microautophagy to reduce presynaptic Tau.

Results: We were able to reduce presynaptic TauP301L levels and restore presynaptic defects by increasing endosomal microautophagy. However, increasing endosomal microautophagy is not effective in lowering TauV337M at presynapses. Using a fluorescent timer attached to Tau, we show that older Tau is present in TauV337M compared to TauP301L mutant presynapses indicating that the turnover of Tau with a disrupted endosomal microautophagy recognition motifs is hindered. In addition, with a fluorescence recovery after photobleaching assay we show that TauP301L is more mobile in axons compared to TauV337M indicating that TauP301L detaches more easily from the microtubuli. Lastly, the expression of Tau containing both P301L and V337M mutations worsens Tau pathology at presynapses.

Conclusions: Together, these data show that both the detachment of Tau from microtubuli and defective turnover of Tau at presynapses contribute to the accumulation of Tau at presynapses and that increasing endosomal microautophagy may be an effective strategy in reducing Tau pathology, at least for pathogenic Tau with intact endosomal microautophagy recognition motifs.
ON-DEMAND SYMPOSIUM: MOLECULAR MECHANISMS IN PROTEINOPATHY, TAUOPATHIES AND SYNUCLEINOPATHIES

SUBCOMMISSURAL ORGAN-SPONDIN-DERIVED PEPTIDE INCREASES THE INTEGRITY OF BLOOD-BRAIN BARRIERS

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Embargo
ON-DEMAND SYMPOSIUM: MOLECULAR MECHANISMS IN PROTEINOPATHY, TAUOPATHIES AND SYNUCLEINOPATHIES

TARGET ENGAGEMENT OF A SUBCOMMISSURAL ORGAN-SPONDIN-DERIVED PEPTIDE ENSURING NEUROPROTECTION AND SYNAPTIC TRANSMISSION IN THE CNS

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Aims: Neurodegeneration and synaptopathy are common features of neurodegenerative diseases which disrupts neural circuits and the execution of motor and cognitive functions. The subcommissural (SCO)-spondin is a brain-specific glycoprotein that contributes to neuronal development. The aim of this study was to investigate the effect of a SCO-spondin-derived peptide (NX210c) on neuroprotection and synaptic transmission and to identify NX210c receptors mediating such properties.

Methods: Cell culture, electrophysiology and in silico methods were used as target engagement assays in parallel.

Results: NX210c promoted neuronal survival against glutamate-induced excitotoxicity on rat cortical neurons by activating the PI3K/mTOR pathway and by disrupting apoptosis. This effect was abolished in presence of an anti-β₁-integrin antibody or an inhibitor of Notch signaling pathway (Delétage et al., 2021). Evoked isolated NMDAr and AMPAr excitatory postsynaptic currents (EPSC) were increased in hippocampal CA1 pyramidal neurons after stimulation of Schaffer collaterals when brain slices were perfused with NX210c. Further patch clamp experiments showed that on contrary to GluN2B, GluN2A subunit of NMDAr triggered the increase of EPS in presence of NX210c. According to the results obtained using cell cultures and electrophysiology, an in silico approach using SUMO® and Rosetta® docking methods confirmed the strong binding of NX210c to β₁-integrin, Notch1, NMDAr GluN2A, and AMPAr.

Conclusions: Collectively, these results show that NX210c represents an innovative drug candidate with a unique multifunctional mechanism of action to treat CNS diseases and injuries; it triggers neuroprotection through its binding to β₁-integrin and Notch, and synaptic transmission through its binding to specific glutamatergic receptors.
ON-DEMAND SYMPOSIUM: MOLECULAR MECHANISMS IN PROTEINOPATHY, TAUOPATHIES AND SYNucleinopathies

LRRK2-G2019S ALTERS DOPAMINERGIC AND ASTROCYTIC DIFFERENTIATION DYNAMICS VIA NR2F1

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Aims: The objective of this study was to investigate how the Parkinson’s Disease (PD)-associated mutation LRRK2-G2019S affects the differentiation dynamics of neurons and astrocytes.

Methods: We used induced pluripotent stem cell (iPSC) lines derived from PD patients carrying the LRRK2-G2019S mutation and we generated 2D cultures of dopaminergic neurons and midbrain organoids (MOs). We then performed single-cell RNA-sequencing (scRNA-seq), ChIP-seq, and immunohistochemical analysis to evaluate the impact of the mutation on the differentiation dynamics. We also used mouse embryos to validate in vivo some of our findings.

Results: LRRK2-G2019S induced accelerated neurogenesis at the expense of astrogenesis in patient-specific 2D cultures and MOs. However, these faster-specified neurons were more prone to undergo cell death. When looking at the potential drivers of these alterations, we identified NR2F1 among the transcription factors of the core regulatory circuits, involved in maintaining the cell identities. Both NR2F1 transcript and protein expressions were severely reduced in astrocytes and neurons carrying the LRRK2-G2019 mutation compared to isogenic controls. To verify the connection between the altered NR2F1 expression and the impaired dopaminergic differentiation, we analyzed Nr2f1 mutant embryos. We observed a significant increase of early dopaminergic differentiation and increased apoptosis when Nr2f1 was downregulated.

Conclusions: Our data uncover a new pathogenic mechanism involving the LRRK2-G2019S mutation, where the dynamics of dopaminergic and astrocytic differentiation are modified via NR2F1.
ROLE OF PRO-INFLAMMATORY S100 PROTEINS IN AMYLOID NEUROINFLAMMATORY CASCADE IN NEURODEGENERATIVE DISEASES.

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Aims: The amyloid cascade is central for the neurodegeneration disease pathology, including Alzheimer’s and Parkinson’s diseases, and remains the focus of much current research. Increasing evidence has accumulated demonstrating critical role of pro-inflammatory S100A9 in the amyloid-neuroinflammatory cascade in these diseases.

Methods: AFM, fluorescence, immunohistochemistry, western blots, ELISA, circular dichroism

Results: We demonstrated that S1009 protein is amyloidogenic and form amyloids both in vitro and in vivo in cell models and in neurodegenerative diseases. In Alzheimer’s, deciphering the interaction between proinflammatory S100A9 protein and Aβ peptide and their co-aggregation mechanisms are particularly important since these lead to amyloid plaques formation and neural cytotoxicity. By using the combination of mass and charge distributions of amyloids together with reconstruction of the differences between them and detailed microscopy reveals that co-aggregation involves templating of S100A9 fibrils on the surface of Aβ amyloids. Kinetic analysis further corroborates that the surfaces available for the Aβ secondary nucleation are diminished due to the coating by S100A9 amyloids, while the binding of S100A9 to Aβ fibrils is validated by a microfluidic assay. We demonstrate that synergy between charge detection mass spectroscopy, microscopy, kinetic and microfluidic analyses opens new directions in interdisciplinary research. Interactions of S100A9 with small molecules as potential regulators of its amyloid aggregation and functions, including interactions with NCAM1 peptide constructs, oleuropein aglycone, polyoxometalates and DOPA-derivatives, are discussed in the light of their potential therapeutic applications.

Conclusions: S100A9-driven amyloid-neuroinflammatory cascade could be a common denominator in a range of neurodegenerative diseases and therefore the common target for therapeutic interventions.
POSTERS

THE MODE OF SYNERGISTIC SUPPRESSION ON LARGE CONDUCTANCE CALCIUM-ACTIVATED POTASSIUM CHANNEL BY AMYLOID BETA OLIGOMER AND AMYLOID PRECURSOR PROTEIN

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Aims: We have previously reported that intracellular amyloid β (Aβ) and amyloid precursor protein (APP) cooperatively suppresses the large-conductance calcium-dependent potassium (BK) channel in cortical pyramidal cells from wild type (WT) mice and triple transgenic AD model (3xTg) mice, and activity-dependent expression of the scaffold protein Homer1a reverses this suppression of BK channel. These findings suggest that Long homer, the splicing variant of Homer1a, is the key molecule, given that it forms 4-mer, can bind BK channel and APP which binds Aβ or APP itself for dimerization. This study further investigated the mode of BK channel blockade synergistically mediated by Aβ and APP.

Methods: Whole cell recordings were performed from the pyramidal neurons in frontal neocortical slices of WT or 3xTg mice. Spike properties were recorded under the intracellular injection of recombinant Aβ1-42, full length APP, Bri2 or various antibodies against APP or Aβ through patch pipette.

Results: BK channel suppression still occur even both in intracellularly APP-injected WT neurons in the presence of 11A1 (Aβ oligomer antibody), and in 3xTg neurons in the presence of Bri2, which disrupt the link between Aβ and APP and the processing of APP to Aβ. In Aβ1-42-injected WT neurons, BK channel suppression failed to be reversed by antibodies against APP.

Conclusions: Considering that the APP C-terminus domain is known to bind to the Homer EVH domain, these observations suggest that long Homer and BK channel, together with APP and Aβ oligomer, may form a molecular complex that underlies synergistic BK channel suppression by APP and Aβ oligomer.
Aims: People with Down Syndrome (DS) carry an extra copy of human chromosome 21 (Hsa21), including amyloid precursor protein (APP). Duplication of APP alone has been shown to be sufficient to cause early-onset Alzheimer’s Disease (AD) (Sleegers et al., 2006). However, the contribution of other Hsa21 gene duplications to AD risk is not yet understood. Using a Trisomic mouse model (Tc1) that lacks functional APP, Wiseman et al., demonstrated that the triplication of Hsa21 genes other than APP increases the risk of AD pathology (Wiseman et al., 2018). We aim to identify which other Hsa21 genes, when expressed at higher levels in people with DS, modify Aβ accumulation or cognitive decline.

Methods: To identify genes that modify Aβ aggregation, we are using the fruit fly Drosophila melanogaster as a screening tool. We will be using two main readouts of increased Aβ accumulation. Firstly, a negative geotaxis assay to assess neumotor function of Aβ expressing flies that overexpress one of the Hsa21 homologues. Secondly, Aβ1-42 levels will be quantified by ELISA. Genes that are found to have an effect on Aβ aggregation in the fly brain will be examined in human post-mortem brain tissue, to determine if they are overexpressed in people who had DS compared with euploid individuals.

Results: We are currently undertaking the screen of 42 genes. The data presented will be that of the current state of the screen.

Conclusions: will be drawn once the screen has been completed.
Aims: The "Unifying Hypothesis of Alzheimer's disease (AD)" is updated based on new data that further demonstrates heparan sulfate proteoglycans (HSPGs) / heparan sulfate (HS) glycosaminoglycans (GAGs) are key to AD pathogenesis and neuropathology as first hypothesized by Snow and Wight over 30 years ago (Neurobiol. Aging 10:481-497, 1989).

Methods: The early accumulation of HSPGs/ HS GAGs links all the observed neuropathology in AD brain (i.e. plaques, tangles and cerebral amyloid) as well as inflammation, genetic factors (involving ApoE), AD-in-a-dish studies, beta-amyloid protein as a microbial peptide, and theories that bacteria, gut microflora, gingivitis and viruses all play a role in the cause of AD.

Results: The common link is the early accumulation of HSPGs/ HS GAGs. HS GAGs and highly sulfated macromolecules induce Aβ 1-40 (but not Aβ 1-42) to form spherical congophilic maltese-cross star-like amyloid core deposits identical to those in AD brain. Heparin/HS induce tau protein to form paired helical filaments. Knockout of HS genes markedly reduce Aβ fibril accumulation in brain demonstrating that HSPGs/ HS GAGs are key. Bacteria and viruses all use cell surface HS GAGs for entry into cells, including SARS-CoV-2. Bacteria and viruses cause HS GAGs to rapidly increase to cause near-immediate aggregation of Aβ into fibrils. Mucopolysaccharidosis (MPS) caused by lack of specific HS GAG enzymes lead to accumulation of Aβ, tau, α-synuclein and PrP in mouse models. Brain aging also leads to changes in HSPGs, including newly identified perlecan splice variants that could increase HS GAG sulfation in AD brain.

Conclusions: All these events demonstrate the new "Unifying Hypothesis of AD".
Aims: Most age-associated neurodegenerative disorders involve the aggregation of specific proteins within the nervous system, as occurs in Alzheimer's disease (AD). Recent evidence indicates that Aβ can misfold and aggregate into seeds that structurally corrupt native proteins, mimicking a prion-like process of template protein corruption or seeding. In fact, studies in animal models show that the injection of brain homogenates from AD patients or from aged APP-transgenic mice containing Aβ aggregates, can induce some of the neuropathological hallmarks of AD. However, it is still unknown which Aβ-misfolded species are most efficient in triggering the aggregation process. Here, we seek to perform a comparative study to determine whether Aβ seeds from humans vs a familial AD line (the 3xTg-AD model) is more efficient to generate amyloid aggregates.

Methods: We employed histological and molecular approaches to determine amyloid level, species and aggregative capacity of brain homogenates from an AD patient (stage C for amyloid, from the Alzheimer's Disease Research Center at UCI) vs old-3xTg-AD mice (25-month-old). Such brain homogenates were injected into the hippocampus of 7-month-old 3xTg-AD mice and the mice were analyzed at 18 months of age.

Results: Our findings demonstrated that amyloid seeds from the human patient have more capacity to generate Aβ plaques vs seeds from aged 3xTg-AD mice.

Conclusions: These results suggest that seeds from human patients seem to be more amyloidogenic than from aged 3xTg-AD mice. Thus, more profound understanding these factors will provide key insight on how amyloid pathology progress in AD.
Aims: Amyloid β (Aβ) can cause DNA damage by inducing ROS, and Alzheimer's disease (AD) patients are known to have a defective ability to DNA repair. However, there have been a few studies on the associations between Aβ levels and DNA repair capacities. Therefore, the purpose of this study was to investigate the effects of short-term beach fitness retreat program (BFRP) on DNA repair activity and Aβ level in middle-aged women.

Methods: Total of forty middle-aged women from age 50-64 were recruited after confirming normal cognitive function by MMSE. And the BFRP is composed of two hours of Nordic walking and another two hours of beach yoga every day during the 5 days of program in Wando, Korea.

Results: DNA repair activity (DRA) increased in twenty subjects and decreased in seventeen subjects. The DRA increase group showed a significant increase of DNA repair activity comparing before and after BFRP (p<.003) with decrease of Aβ levels from 0.6965±0.04 to 0.6624±0.05 (Table 1). The Pearson correlation coefficient between the change of DRA and Aβ levels in this group was 0.402 (p=0.098) in Fig 1. However, the DRA decrease group showed a significant decrease of DNA repair activity comparing before and after BFRP (p<.007) and showed increase of Aβ levels from 0.6413±0.08 to 0.7266±0.02 with insignificant correlation (r=-0.145, p=0.621).
Conclusions:
The short-term BFRP had a possible positive effect to reduce Aβ in DRA increase group in middle-aged women and it is needed to evaluate the association between Aβ levels and DRA in larger study subjects.

Table 1. Changes of DNA repair activity and Amyloid β after 5 days of fitness retreat program

<table>
<thead>
<tr>
<th>Factor</th>
<th>Pre-</th>
<th>Post-</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA repair activity (%)</td>
<td>-49.62 ±30.94</td>
<td>62.65 ±10.96</td>
<td>0.003**</td>
</tr>
<tr>
<td>Amyloid β</td>
<td>0.6965 ±0.64115</td>
<td>0.6624 ±0.04771</td>
<td>0.570</td>
</tr>
<tr>
<td>DNA repair activity (%)</td>
<td>138.46 ±47.21</td>
<td>-9.76 ±22.97</td>
<td>0.007**</td>
</tr>
<tr>
<td>Amyloid β</td>
<td>0.6413±0.08</td>
<td>0.7266 ±0.01648</td>
<td>0.273</td>
</tr>
</tbody>
</table>

Values are Mean±SD
Plasma amyloid β was measured using the MDS (Multimer detection system).

Figure 1. Correlation with DNA repair activity and Amyloid β.
A. Scatter plot of DNA repair activity (DRA) increase group. Increases in change of DNA repair activities were weakly correlated with decrease of amyloid β (r=0.402, p=0.098, n=18). B. Scatter plot of DRA decrease group. The regression line shows an inverse correlation that is not statistically significant (r=-0.145, p=0.621, n=18)

Conclusions: The short-term BFRP had a possible positive effect to reduce Aβ in DRA increase group in middle-aged women and it is needed to evaluate the association between Aβ levels and DRA in larger study subjects.
DEVELOPING BETA-SECRETASE INHIBITORS FOR TREATMENT OF ALZHEIMER’S DISEASE

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Aims: AD is defined as a neurodegenerative disorder accompanied by progressive memory loss which is affiliated with many cognitive complications. BACE inhibitors are in priority for drug designing of AD. Production of amyloid-beta is initiating with amyloid-beta production therefore its suppressing can be an ideal target for therapy of Alzheimer’s disease. In this paper, AD pathophysiology, beta-secretase structure, BACE1 classification, and their correlated adverse and beneficial effects as well as BACE1 inhibitors that are being investigated in clinical trials like LY2811376, LY3314814 (AZD3293), CNP520, Elenbecestat (E2609), Mk8931 (Verubecestat), LY2886721. Symptomatology AD treatment techniques Drug design strategies Classification of BACE inhibitors BACE inhibitors toxicity and selectivity Success and failure results correlated with BACE1 inhibitors using the statistical method

Methods: We designed a statistical model to simulate the success and failure rates using different BACE1 inhibitors in mice and human treatment. We used maximum likelihood estimation to model AD progression rate after using different inhibitors over using past 10 years data in both human and mice.

Results: The result shows significant differential effectiveness between mice and humans. The statistical model shows acceptable results compared to the real cases. The results can be utilized to utilizing computer-aided drug design.

Conclusions: The capability of BACE1 to apply such a therapeutic candidate for AD therapy has just been examined during the previous decade. There is proof indicate that the 1 inhibitor administrating time is critical and make big difference in how successful they are in curing AD.
MEMBRANE COMPOSITION CAN MODULATE CELLULAR RESILIENCY TO AMYLOID-BETA42 OLIGOMER TOXICITY

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Aims: Protein misfolded oligomers comprised of the 42-residue form of the amyloid-β peptide (Aβ42) are thought to play a key role in the onset and development of Alzheimer’s disease. These metastable and heterogenous aggregates interact with a wide range of phospholipids and receptors in the cellular membrane, therein disrupting membrane integrity and ion homeostasis. A more detailed understanding of the nature of this deleterious interaction would give valuable insight into drug discovery targets within the cell membrane to combat oligomer toxicity.

Methods: Using cellular assays to quantify the integrity and viability of SH-SY5Y human neuroblastoma cells, we examined the resiliency of cells enriched with differing concentrations of key membrane lipids to Aβ42 oligomers, such as cholesterol, sphingolipids, ceramides, and other integral molecules. Additionally, we examined the effectiveness of a steroid polyamine countermeasure on cells with differing membrane compositions in neurotoxic conditions.

Results: As monitored using MTT assays and confocal microscopy, we observed changes in the toxicity of Aβ42 oligomers upon cellular enrichment in specific membrane lipids, indicating that Aβ42 oligomers may interact preferentially with certain regions of the cellular membrane. Moreover, we observed that a brain permeable aminosterol could attenuate the toxicity of the oligomers, a finding that was largely independent of the lipid enrichment conditions.

Conclusions: These findings indicate that a molecular countermeasure which competes with oligomers for interactions in certain regions of the plasma membrane may be effective at preventing cell death in Alzheimer’s disease.
Aims: A growing body of evidence suggests that amyloid-beta oligomers (AβO) play a key role in the physiopathology of Alzheimer's disease (AD). Even though aging is the main risk factor for AD, little is known about the susceptibility of aging brain for AβO neurotoxicity. Here, we compared the behavioural and biochemical effects of AβO in both young and aged mice.

Methods: AβO were prepared in-house from human Aβ1-42 monomers. The oligomeric preparations were characterized by SDS-page and dot-plot assays. In vitro, AβO induced a reproducible neurotoxic effect on primary neurons, while monomers did not induce any neuronal injury. In vivo, young (3-months-old) and aged (18-months-old) wild-type mice received a single intracerebral injection of AβO. Memory performances and hippocampal protein levels were assessed for 2 weeks.

Results: Naïve old and young mice had similar memory performances despite significantly lower synaptic marker expressions in aged mice. AβO induced significant synaptic losses in both young and aged mice, but only aged mice showed significant memory impairments in episodic and spatial memory tasks. Interestingly, AβO produced more cerebral inflammation in aged mice than in young mice and promoted neuronal apoptosis as well as functional alterations of astrocytes. Cognitive symptoms in aged mice were reversed by Donepezil.

Conclusions: In conclusion, aging dramatically changes the susceptibility for AβO neurotoxicity in mice. It confirms that the specificities of the aged brain should be considered to improve the translational value of AD models. Our non-inherited AD model is a new tool for preclinical testing of both disease modifying and symptomatic drugs.
THE ROLE OF CELL STRESS IN DRIVING PROTEIN AGGREGATION WITHIN CONTROL IPSC-DERIVED NEURONS

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Aims: Increasing evidence suggests Alzheimer's disease (AD) pathogenesis is not limited to the neuronal compartment but strongly interacts with immunological mechanisms in the brain. There are many factors causing neuronal stress and producing a cytotoxic environment, such as endoplasmic reticulum stress and release of proinflammatory cytokines like Interleukin-1 beta (IL-1β). This can sequentially cause intracellular changes to highly sensitive neurons, leading to misfolding and aggregation of amyloid-beta (Aβ), tau and alpha-synuclein (αSyn) proteins and produce neurotoxic species that leads to neuroinflammation and neuronal death. This study uses normal neurons under different physiological stressors to evaluate how they disrupt protein homeostasis and lead to protein aggregation in a sporadic AD-like manner. To achieve this, aggregates formed within the cell and released in the secretome over time were assessed.

Methods: Control iPSC-derived neurons at early maturation stages were exposed to cell stressors such as thapsigargin, tunicamycin and human IL-1β for 20 days, to determine their influence on the number of Aβ and αSyn aggregates released by neurons as well as formed inside the cells. Cell culture media was collected every 5 days and lysates were collected every 10 days to observe aggregate changes and the level of cell death.

Results: Preliminary data have shown 100 ng/mL tunicamycin and 10 ng/mL IL-1β were able to induce an abnormal release of Aβ and αSyn aggregates in day 70 neurons.

Conclusions: Understanding what stresses cause the release of toxic oligomers will provide more insight into what can cause or contribute to sporadic AD and help find therapeutic treatments.
RT-QUIC IDENTIFICATION OF ALPHA-SYNUCLEIN CO-PATHOLOGY IN ALZHEIMER’S DISEASE

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Aims: Alpha-synuclein co-pathology may influence Alzheimer’s disease (AD) phenotype but its presence has never been tested in large series of patients. Real-time quaking-induced conversion (RT-QuIC) recently provided evidence of alpha-synuclein seeding activity in CSF and olfactory mucosa of patients with alpha-synucleinopathies. Aim of the study was to evaluate the prevalence, biological and clinical correlations of alpha-synuclein co-pathology in patients with Alzheimer’s disease.

Methods: a preliminary set of 82 AD patients (mean age 70.3 ± 7.3 years) underwent CSF analyses for Tau, P-tau and A-beta amyloid and an extensive cognitive, behavioral and motor assessment. RT-QuIC for alpha-synuclein was performed on CSF samples.

Results: alpha synuclein RT-QuIC resulted positive in thirty-three out of 82 AD patients (40.2%). AD patients with positive alpha-synuclein were comparable for age and disease severity those negative to RT-QuIC assay including Tau/P-Tau and A-beta CSF markers in cross-sectional analyses.

Conclusions: these preliminary findings indicate that alpha-synuclein co-pathology might be detected in up to one third of Alzheimer’s disease patients. These findings might potentially explain the clinical variability of AD including age at onset, progression and response to treatment observed in clinical series. Larger longitudinal studies are warranted to confirm and extend these findings.
ANALYSIS OF BETA-AMYLOID CYTOTOXICITY AS A FUNCTION OF STRUCTURE AND AGGREGATION CONDITIONS

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Aims: Beta-amyloid aggregation is proposed to play a crucial role in the pathogenesis of Alzheimer’s disease (AD). Data from several in vitro studies suggest that the aggregates of amyloid-β peptide (Aβ) show a wide range of structural diversity. These structures can be formed simultaneously and even have the tendency to convert into each other, making their characterization even more challenging. Our aim was to investigate how the aggregation condition influences the overall structural composition and toxicity of Aβ42 aggregates. We also addressed in the comparative study the potential use of mHippoE-14, an immortalized embryonic mouse hippocampal cell line as model for Aβ cytotoxicity studies.

Methods: Recombinant Aβ42 was expressed in E. coli BL21 cells. The peptide was aggregated under wide range of conditions: pH 5.5-8.5, 0-500 mM NaCl, 4-50 ºC with or without shaking. The structural composition of the samples was analyzed by ThT fluorescence measurements, transmission electron microscopy, and circular dichroism spectroscopy. Their toxicity was assessed by using immortalized and primary rodent cell lines.

Results: The widest structural variety was found among aggregates formed under physiological-like pH. Higher amyloid content resulted in lower cytotoxicity. We are the first to report that mHippoE-14 cell line showed sensitivity to toxic Aβ42 aggregates and interestingly, pH 8.5 aggregates were more toxic to these cells, while pH 7.5 samples decreased the viability of SH-S5HY cells more.

Conclusions: Our results can help to understand the relationship between Aβ42 structure and toxicity, and also provide support for AD researchers in choosing the right aggregation and testing conditions.
SIZE DISTRIBUTION ANALYSIS OF SOLUBLE OLIGOMERIC SPECIES BY ASYMMETRIC-FLOW FIELD-FLOW FRACTIONATION OF BRAIN HOMOGENATES FROM ALZHEIMER'S DISEASE PATIENTS

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Aims: In a recent analysis of prion oligomers in prion disease, we correlated disease phenotype and duration with prion aggregate size distribution in the brain (Cortez et al, PLoS Pathogens, 2021). To determine whether such protein aggregate size distributions may correlate with phenotypes and durations of Alzheimer’s disease, we adapted our protocol for amyloid beta and tau aggregates from Alzheimer’s disease brains.

Methods: Pathologically-confirmed Alzheimer’s disease brain homogenates were clarified by centrifugation at low speed, then enriched for amyloid-beta (Aβ) and tau aggregates by ultracentrifugation at 100,000 x g for 1h. The pellet (P2) was primarily composed of misfolded proteins and was free of soluble counterparts as demonstrated by Western blotting. To further separate the hyperphosphorylated tau and Aβ, the P2 was treated with different concentrations of sarkosyl. After solubilization, the tau and Aβ preparations were subjected to asymmetric-flow field-flow fractionation (AF4). The hydrodynamic radius, shape and number of eluted particles were measured by in-line dynamic scattering and multi-angle light scattering (DLS/MALS) detectors. The relative amounts of hyperphosphorylated tau and Aβ in each fraction was determined by Western blotting.

Results: We were able to successfully enrich and selectively isolate misfolded tau and Aβ from their soluble counterparts. Using AF4 with in-line DLS/MALS we were then able to characterize the size distributions of each protein aggregate.

Conclusions: We believe this method for isolating and characterizing oligomeric protein species can help correlate oligomeric size distributions with clinical phenotypes of disease, not just for prion and Alzheimer’s disease, but potentially for all protein folding neurodegenerative diseases.
SYNTHETIC AND NATURAL CONFORMATIONAL VARIANTS OF MISFOLDED Aβ INDUCE DIFFERENT PATHOLOGICAL FEATURES IN SUSCEPTIBLE MICE.

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Aims: Compelling evidence in humans and experimental rodents suggest that Alzheimer’s disease (AD)-associated Aβ exists in a variety of conformational strains. The biological significance of Aβ strain variation in AD has not been addressed. This acquires relevance considering that mixtures of Aβ strains seems to exist in the brains of patients. Aβ strain variation may explain pathological and clinical differences observed among people afflicted by AD.

Methods: Here, we used brain-derived and synthetic Aβ strains to assess for potential differences in propagation and pathological manifestations.

Results: In a first set of experiments, two synthetic-Aβ₄₀ strains (2F and 3F) that have been thoroughly studied for their structural motifs, were biochemically characterized and injected in the brains of 50 days-old Tg2576 mice. We assessed prion-like transmission of these materials by analyzing Aβ deposition 250 days later. A second set of experiments involved the administration of AD brain homogenates from individuals displaying diverse amyloid pathology. These brain extracts were intra-cerebrally injected into 30 days-old APP/PS1 mice that were sacrificed 150 days later. Pathological differences in both experiments were found at different levels, including the type and anatomical distribution of the aggregates, Aβ₄₀/Aβ₄₂ ratios, reactivity of amyloid deposits to dyes able to discriminate among misfolded protein conformations, among others. Importantly, differences in astro- and micro-glial activation were also observed.

Conclusions: Our data support the concept and biological relevance of conformational strain variation in non-prion protein misfolding disorders. Our findings may help to identify the most deleterious particles responsible for AD and design conformation-specific strategies for diagnosis and treatment.
Aims: Sleep is vital for brain function. The biological necessity for it might reflect that sleep promotes activity of glymphatic system, which eliminates extracellular metabolic waste products, including amyloid beta (Aβ). Clearing of extracellular proteins prevents their accumulation in the brain parenchyma ensuring tissue homeostasis. In contrast, sleep disturbances reduce glymphatic influx of cerebrospinal fluid (CSF) and elevate risk of protein aggregation. Therefore, it is paramount for the brain to maintain its glymphatic clearance functional.

Methods: We investigated how Aβ efflux dynamics change as a consequence of sleep deprivation in young wild type (WT) mice and in a model of beta-amyloidosis (APP/PS1). Aβ peptide was quantified by commercially available high-sensitivity enzyme-linked immunosorbent assay (ELISA). Our goal was to compare the relative contribution of various glymphatic efflux routes to Aβ clearance – including efflux into CSF, blood and cervical lymph nodes. Ultimately, we aimed to define the role of glymphatic system in the complex process of Aβ efflux from the brain into the periphery.

Results: Preliminary results indicate that sleep deprivation caused a delay of parenchymal soluble Aβ clearance into CSF in WT mice. Furthermore, the rodent model of beta-amyloidosis displayed major efflux disturbances which may hint towards previously unexplored early age glymphatic disfunction.

Conclusions: Quality of sleep is disrupted in patients with Alzheimer’s disease. Stagnant protein metabolites in the brain interstitial fluid can trigger neuroinflammatory responses which may potentiate neurodegeneration. Hence, it is crucial to elucidate how sleep loss alters not only production but also clearance of Aβ in early stages of Alzheimer’s disease.
DEFICIENT COGNITIVE AND EMOTIONAL FUNCTIONS AND NEUROTRANSMITTER LEVELS IN 
THE TGDIMER MOUSE THAT EXPRESSESAMYLOID-BETA DIMERS, BUT NO PLAQUE 
PATHOLOGY

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Aims: Soluble amyloid-beta oligomer has recently been implicated as a critical pathogenic feature in 
Alzheimer's disease (AD), preceding significant amyloid beta-peptide aggregation. Different forms of 
soluble amyloid-beta species are associated with synaptotoxicity and gradual escalation of the clinical 
progression. Although the exact underlying mechanism is still poorly understood, it leads to the known 
early Alzheimer’s synaptic dysfunction, learning and memory deterioration.

Methods: A novel genetically modified “tgDimer” mouse, with reliable production of high amount of a 
single soluble amyloid-beta oligomer “dimer” is investigated. Evidence was obtained for its age-related 
impact on spatial learning, attention, spatial memory synaptic-plasticity and neurochemistry balance. In 
this study, together with further detailed neurochemical characterization of the “tgDimer” mouse by high-
performance liquid chromatography, a comprehensive battery of behavioral tests are carried out to 
assess dysfunction of region-specific learning and memory formation and emotional behaviors.

Results: Our results suggest that amyloid-beta dimer; in the absence of insoluble aggregates, plays a 
significant role in causing deficits in reference, but not working spatial memory, resistance to extinction, 
reward-motivated learning, cognitive flexibility, temporal order memory (what and when), and higher-order 
memories (episodic-like memory). Furthermore, amyloid-beta dimer influences affective symptoms as 
hyponeophagia and anhedonia reminiscent of early AD and the balance of neurotransmitter systems.

Conclusions: The tgDimer mouse allows a more specific description of behavioral phenotypes in early 
AD, where therapeutic approaches are more likely to be effective before the massive structural changes 
occur. We conclude that amyloid-beta dimers contribute to neurotransmitter dysfunction and behavioral 
impairments, characteristic of the early stages of AD.
AN IN VITRO PLATFORM FOR MODELING AND MEASURING THE OLIGOMER STATE IN THE AMYLOID B GROWTH DYNAMICS

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Aims: A variety of different amyloid β (Aβ) protein aggregates forms in Alzheimer’s disease (AD), and this aggregation pathway has been linked with severity of AD. Therefore, monitoring the process of Aβ aggregation could facilitate the identification of pathogenic indicators that enable early-stage diagnosis and treatment.

Methods: We show a strategy for label-free terahertz (THz) optical monitoring the aggregation dynamics of Aβ proteins—from monomers to fibrils—under physiological conditions including body temperature and incubation medium (e.g., buffer versus Matrigel) with a nanomolar detection limit using near-field THz spectroscopy.

Results: We clearly reveal the rate-limiting, stepwise nature of this aggregation process, identifying three steady states for polymerization of monomers, oligomers, and fibrils, separated by two transition states. We verify the existence of these states by analyzing sample morphologies with atomic force microscopy. We further show that the transition time and growth rate of each steady state vary with physiological conditions and are modeling a universal equation for full growth pathway of Aβ aggregation.

Conclusions: We anticipate that our method for the label-free detection of Aβ oligomer state could ultimately facilitate the earlier clinical diagnosis of AD with drop-sized physiological samples.
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**Aims:** Alzheimer’s Disease (AD) is characterized by two pathological signs, amyloid plaques composed of aggregations of proteolytic products of Amyloid Precursor Protein (particularly Amyloid-β, Aβ) and neurofibrillary tangles (NFTs) composed of hyperphosphorylated microtubule-associated protein tau. Whereas plaques are initially found in parietal neocortex and spread throughout the cortex, the neurodegeneration associated with NFTs starts in particular neurons in the superficial layers of the entorhinal cortex (EC). NFTs then spread through the EC, then to the hippocampus, subiculum, and eventually neocortical areas. This suggests EC layer II neurons are particularly vulnerable to the pathological interaction between tau and Aβ that underlines AD pathogenesis.

**Methods:** To investigate this, we injected large volumes of an AAV vector expressing wildtype human-Tau via the Synapsin promoter near the rhinal fissure of 1-month-old rats expressing familial AD mutations (McGill-R-Thy1-APP rat model). Since these rats express Aβ throughout the telencephalon, this allows us to study the interactions between overexpression of wildtype human Tau and Aβ in a variety of neuronal cell types throughout EC and adjacent cortex.

**Results:** Injections led to greatly increased Tau hyperphosphorylation relative to controls (wildtype rats and those injected with AAVs expressing murine Tau) as judged by the expression of a variety of phospho-Tau antigens. Moreover, Tau hyperphosphorylation was disproportionately found in a particular subset of ECLII projection neurons positive for Reelin (i.e. stellate cells).

**Conclusions:** This suggests that these neurons have a predilection for the pathological interactions between human-Aβ and Tau leading to AD, providing an anatomically-correct model to study the earliest stages of its pathogenesis.
INHIBITION OF CPLA2 AMELIORATES NEUROINFLAMMATION IN APOE4

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Aims: Apolipoprotein E4 (APOE4) is associated with a greater response to neuroinflammation and the risk of developing late-onset Alzheimer's disease (AD), but the mechanisms are not clear. The activation of calcium-dependent cytosolic phospholipase A2 (cPLA2) is involved in inflammatory signaling. We previously reported higher cPLA2 activation and its related pro-inflammatory lipid metabolites with APOE4 in human brains, primary astrocytes, and brains of ApoE-targeted replacement (ApoE-TR) mice compared to APOE3. It's unknown whether inhibition of cPLA2 decreases ApoE4-induced neuroinflammation.

Methods: First, younger ApoE4 mice (4 months old) fed a chow diet were administrated daily with cPLA2 inhibitor (ASB14780, 10mg/kg, intraperitoneal) for three days. The brains were collected and the lipidomics were analyzed by GC-MS. In a second experiment, older ApoE4 mice (n=17, 16 months old) fed a low-DHA diet were injected with the cPLA2 inhibitor daily (ASB14780, 10mg/kg, intraperitoneal) for three weeks, and followed by behavioral tests, pathological, lipidomic and biochemistry assays, and PET MRI imaging of 18-F DHA and EPA uptake, together with measures of blood-brain barrier integrity using DCE MRIs.

Results: Here, we found that treatment with the cPLA2 inhibitor ASB14780 penetrated the brain and altered the brain lipidome in ApoE4 mice (4 months old, chow diet), decreasing the pro-inflammatory metabolites of arachidonic acid. Treatment with ASB14780 for 3 weeks decreased brain proinflammatory lipids and increased both DHA and EPA levels in the brains (p<0.001). Moreover, deficits of recognition and memory of older ApoE4 mice were non-significantly ameliorated (p=0.15) after treatment. Brain imaging analysis is ongoing.

Conclusions: Our findings implicate targeting cPLA2 signaling system with APOE4, for reducing the increased neuroinflammation with APOE4 and AD.
BNIP3 SELECTIVELY EXPRESSES IN ENTORHINAL CORTEX LAYER II-NEURONS THAT ALSO EXPRESS REELIN

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Aims: Characterization of Bnip3 expression in rodent and human brain.

Methods: Immunohistochemistry, western blotting

Results: In a rat model for AD (McGill-R-Thy1-APP model) expressing human mutated APP, experiments revealed that in EC, Reelin-expressing layer II neurons selectively stain positive for intracellular Aβ at the pre-plaque stage, and this might hold true also for early-stage AD subjects (Kobro-Flatmoen et al., 2016). Here we show that this population also selectively expresses Bcl-2/adenovirus E1B 19KDa-interacting protein (Bnip3).

Conclusions: The selective expression of Bnip3 in EC layer II-neurons is of interest since Bnip3 participates in mitophagy but can under certain conditions cause caspase-independent neuronal death.
TEMPORAL CHANGES IN PERSONAL ACTIVITY INTELLIGENCE AND RISK OF DEMENTIA INCIDENCE AND MORTALITY: A POPULATION-BASED PROSPECTIVE COHORT STUDY

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Aims: While numerous studies suggest physical activity (PA) to be beneficial for dementia risk, only a few studies analyze what PA measure is most indicative of being beneficial. We aimed to investigate the association between temporal changes in physical activity over two decades with risk of incident dementia and dementia-related mortality.

Methods: We used personal activity intelligence to quantify physical activity (PAI scores) in participants from two waves of the Nord-Trøndelag Health Study (HUNT1: 1984–1986, and HUNT2: 1995–1997, n=29826). This was linked to data on incidence from the Dementia Registry (2011-2021, 1998 participants) and the Norwegian Cause of Death Registry (from HUNT2 until 2020 or death, 1033 cases).

Results: Those with a persistently high PAI score (≥100 PAI at both time points) had 18% (95% CI: 1-31%) lower risk of incident dementia and 27% (95% CI: 4-44%) lower risk of dementia-related mortality, when compared to those with persistently low PAI scores. Those who improved PAI scores over time (from <100 to ≥100 PAI) had 17% (95% CI: 4-28%) lower risk of incident dementia and 26% (95% CI: 8-41%) lower risk of dementia-related mortality, when compared to the reference group with constant low PAI scores.

Conclusions: Individuals attaining a weekly score of ≥100 PAI have lower risk of dementia incidence and dementia-related mortality. This knowledge may offer a window of opportunity to intervene early, in particular for Alzheimer’s disease which has a several decades long preclinical phase and constitutes 70% of all dementia cases.
An In Vitro Platform To Study Adult Entorhinal Networks In Alzheimer’s Disease

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Aims: Entorhinal cortex (EC) layer II contains the first affected cortical neurons in the course of Alzheimer’s disease, suffering extensive early tangle pathology and neuron loss. We recently showed that these neurons express the glycoprotein reelin, and substantiated the finding that these neurons originate the exclusive projection to the hippocampal dentate gyrus and CA3. These reelin expressing EC layer II-neurons align along a gradient where those located successively closer to the rhinal fissure express increasingly higher amounts of reelin. Intriguingly, these neurons selectively accumulate intracellular amyloid-β and this accumulation aligns along the same gradient.

Methods: We are developing a platform to study these neurons at adult stages in vitro. To this end we surgically extract and culture EC layer II-neurons from both young adult APP/PS1 model mice, and McGill-R-Thy1-APP model rats, all on microelectrode arrays to enable electrophysiological recordings of network activity.

Results: Our long-term aims are to investigate early cell- and network-level dysfunction hypothesized to arise during the early build-up of intracellular amyloid-β, and to determine factors that may lead to cell death and spread of the disease.

Conclusions: First results show survival of reelin expressing EC layer-II neuron cultures up to 60 days in vitro, with structural connections in place after 3 days in vitro. We detect spiking activity coincident with local field potentials in the network after 14 days in vitro.
Aims: All disease-targeting drug trials completed to date have failed to meet the clinical endpoint of significantly slowing cognitive decline in Alzheimer’s disease patients. Even the recently approved drug, Aducanumab, has proven effective in removing amyloid-β, but does not reduce cognitive nor functional decline. This emphasizes the urgent need for novel therapeutic approaches that reduce several AD neuropathologies simultaneously, eventually leading to improved cognitive performance.

Methods: In this study we have repurposed two drugs, Fasudil and Lonafarnib, with the potential to target several aspects of AD pathology at once. Furthermore, these drugs are already approved for other medical causes in patients, and their safety confirmed by previous studies.

Results: Using intracerebral microdialysis for the simultaneous infusion of disease-modifying drugs and collection of cerebrospinal fluid, we found that Fasudil reduces intracellular amyloid-β in young animals, and amyloid plaques and cerebrospinal fluid amyloid-β in old animals, while Lonafarnib reduces tau neuropathology and cerebrospinal fluid tau biomarkers in young and old animals. However, an unexpected finding was that Lonafarnib treatment increased amyloid plaques and cerebrospinal fluid amyloid-β in old animals, suggesting that activating the endosomal-lysosomal system may inadvertently increase amyloid-β pathology if administered too late in AD. Co-infusion of both drugs appears to attenuate contextual memory deficits, compared to mice receiving a vehicle.

Conclusions: Taken together, these findings lend support to the application of repurposed drugs to attenuate Alzheimer’s disease neuropathology at various time points in preclinical models to probe potential biochemical events that result in Alzheimer’s disease.
A SWITCH FOR ALZHEIMER’S DISEASE NEUROPATHOLOGY AT ITS REGIONAL ORIGIN

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Aims: A major unresolved issue within the Alzheimer’s disease (AD) field includes how neuronal circuits become dysfunctional in response to AD-related neuropathology and how circuit abnormalities can be repaired. The lateral regions of superficial layers in lateral EC entorhinal cortex (LEC LII) is where neuropathology likely begins in AD in terms of neurofibrillary tangles. To determine how particular EC cells may be implicated in preclinical AD and to develop interventions to stop the disease process in these cells, we hypothesized that the projections from LEC LII to the hippocampus might promote a dysfunctional spread of AD neuropathology via excitatory projections.

Methods: Here we initiated pathology by over-expressing mutated human tau (huTau), and chemogenetic receptors in combination with a novel DREADD ligand to chronically inhibit LEC LII neurons to examine the effect on neuropathological development in the hippocampus of 3xTg AD mice.

Results: Our findings indicate that the viral-mediated over-expression of huTau into LEC LII resulted in propagation of huTau from LEC LII to the hippocampus. After longitudinally silencing LEC layer II neurons using hM4 DREADDs we found that AD neuropathology was attenuated in downstream hippocampus, and lead to impairment in a contextual memory task, highlighting the role of the EC-hippocampal network in this type of memory formation.

Conclusions: In conclusion, we have obtained new insights into the origins and mechanisms of neuropathological spread in AD and demonstrate for the first time that amyloid pathology may be spreading in a similar manner to tau during development of the disease.
Aims: To evaluate the presence of misfolded α-synuclein (αSyn) in the cerebrospinal fluid (CSF) and αSyn aggregates in postmortem brain tissue of patients with Autosomal dominant Alzheimer's disease (ADAD).

Methods: 18 CSF samples from ADAD symptomatic patients were tested for αSyn using Real-time quacking-induced conversion (RT-QuIC) technique. Postmortem brain tissue from 21 ADAD brain donors was studied. We applied NIA-AA consensus criteria for AD pathology staging, McKeith system for αSyn deposits and LATE-NC stages for TDP43 pathology.

Results: Mean age of CSF samples was 49±9 years with 10/18 females (56%). Clinically patients were in mild (7/18) and moderate stages of the disease (11/18). Mean age of brain donors was 56±9 years with 4/21 females (19%), all in advance clinical stages of AD. Amplification of αSyn was observed in 1 patient (6% of cases). AD staging revealed an A3B3C3 score in 19 subjects (90%) and an A3B2C3 score due to Braak IV in 2 subjects (10%). αSyn aggregates were present in 9 subjects (43%): 5 presented amygdala predominant deposits (56%), 2 limbic (22%), 1 neocortical and 1 in olfactory bulb only. In addition, TDP43 concomitant pathology was observed in 2 patients (10%) (LATE-NC stage 2).

Conclusions: The presence of misfolded αSyn in CSF is scarce in symptomatic ADAD, in contrast to the presence of brain aggregates in postmortem tissue. This fact might be related to the late appearance of concomitant αSyn pathology in the course of the disease or to the distribution and/or to the strain of the αSyn deposits in the brain.
NEUROINFLAMMATORY ALTERATIONS ASSOCIATED WITH INTRANEURONAL AMYLOID-BETA IN EARLY ALZHEIMER’S DISEASE

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Aims: The overall aim of this research is to investigate what, if any, neuroinflammatory alterations are occurring in relation to intraneuronal amyloid-beta (iAβ) pathology in early Alzheimer’s disease (AD). Specifically, we aim to determine whether neuronal and microglial markers and morphology and neuron-microglia interactions and signaling are altered in association with iAβ.

Methods: To study this, we utilized the 5xFAD mouse model of AD at ages before and at the onset of plaque development. Utilizing the anatomical pathway between the subiculum (SUB) and mammillary bodies (MB), we could parse out somatodendritic versus axonal Aβ and alterations associated specifically with each. We then performed immunohistochemistry and probed for markers that are known to be involved in neuronal and microglial health and function and neuron-microglia interactions.

Results: In young, pre-plaque 5xFAD AD-model mice, we observed punctate and wispy plaque-like aggregated iAβ in SUB and MB, respectively. Despite strong Aβ signal and the presence of Aβ resembling early plaques, no gross microglial alterations, such as those associated with plaques, were observed in association with the Aβ in SUB nor MB. We did, however, observe microglial uptake of Aβ42 near the plaque-like structures in MB.

Conclusions: Our current understanding of AD is framed around the amyloid cascade hypothesis, which supposes that extracellular Aβ plaques trigger downstream events, including intracellular tau aggregation, neuroinflammation and cognitive decline. However, increasing evidence, including our work, is showing how, even before plaques, neuroinflammatory and synaptic alterations as well as aggregation of iAβ are already occurring, likely setting the stage for later pathological events.
INCREASED EXPRESSION OF SUPPRESSOR OF CYTOKINE SIGNALING PROTEIN 3 IN ALZHEIMER’S DISEASE

Aims: Suppressor of cytokine signaling (SOCS) proteins are master regulators of the cells’ response to cytokines that would play an important role in the inflammatory response associated with AD, as well as in the amyloid processing and deposition. We aim to study whether the expression of SOCS1 and SOCS3 proteins are altered in Alzheimer’s disease.

Methods: We have studied the expression of SOCS1 and SOCS3 by western blot and qPCR in the brain cortex of Alzheimer’s patients, from early (I-II) to late (V-VI) Braak & Braak stages. We have also analyzed in BV2 microglial cell line, SOCS3 and SOCS1 response to the activation by lipopolysaccharide as compared with that exerted by amyloid-β.

Results: Our study shows that SOCS3 protein and transcript levels are increased in AD brains. This increment occurs since first stages of the pathology and is maintained until late Braak V-VI stages. However, the levels of SOCS1 remains unchanged in AD samples. LPS activation increases SOCS3 levels, with no changes in SOCS1 expression. Moreover amyloid-β stimulation shows microglial-like activation with increased SOCS3 response but not of SOCS1. We also provide results of the presence of SOCS3 in human microglia and neurons derived from induced Pluripotent Stem Cells from Alzheimer’s patients and control subjects.

Conclusions: In Alzheimer’s disease brain’s there is an increase in SOCS3 levels that could reflect the microglial activation as result of amyloid-β stimulation.
Aims: Many reports have shown an excessive synaptic loss in Alzheimer's disease (AD) mouse models that is dependent on the complement system at the synapses. Here, our goal is to test whether C5a-C5aR1 signaling is actively contributing to synaptic engulfment by microglial cells in two different mouse models of Alzheimer's disease, and thus, is contributing to the excessive synaptic loss.

Methods: Immunofluorescence with markers for microglia, lysosomes and synapses were assessed in brain sections of WT, C5aR1KO, Arctic and Arctic-C5aR1KO mouse models at different ages; as well as in the Tg2576 mouse model of AD treated with a C5aR1 antagonist (PMX205) for 12 weeks. Imaris 3D reconstruction analysis and quantification were carried out to test differences in microglial activation and phagocytosis.

Results: 3D analysis of microglial cell synaptic engulfment showed significant differences at 10m in two different hippocampal regions (CA1 and CA3) in the Arctic-C5aR1KO compared to the Arctic mice. Furthermore, PMX205 treatment in 12-month-old Tg2576 mice also showed a significant reduction in synaptic pruning, that correlated with a rescue in VGLUT1 presynaptic loss.

Conclusions: Our results suggest that in later stages of Alzheimer's disease, C5aR1 (either by genetic ablation or pharmacological inhibition) influences microglial synaptic pruning and that this contribution is age and regional dependent. While the mechanisms underlying this remains elusive, pharmacological modulation of C5a-C5aR1 signaling could be a potential therapeutic target to treat this neurodegenerative disease.
Aims: Alzheimer’s disease (AD), the primary cause of dementia, disproportionally affects women for reasons that are not well understood. Robust neuroinflammation in the brain and its interfacing regions is a hallmark of the disease and exhibits sex differences. Whether these differences are due to gonadally-derived hormones or the X chromosome – itself encoding many immune-related genes, is unclear, as is whether these differences contribute to sex differences in disease onset and progression.

Methods: To dissociate gonadal effects from the sex chromosome, we used the Four-Core Genotype (FCG) mouse model that produces testes- or ovary-bearing mice with either XX or XY sex chromosomes. FCG mice were crossed to the 5xFAD mouse model, and offspring were aged to assess the impact of gonads and sex chromosome complement on disease hallmarks.

Results: Aged 5xFAD; FCG mice exhibited chromosome-specific changes in meningeal amyloid-beta accumulation and gene expression changes in the choroid plexus. Furthermore, in the brains of these animals, we found differential effects of the gonads and sex-chromosome complement on genes frequently associated with amyloid pathology.

Conclusions: Our data suggest that gonadally-derived hormones and sex chromosomes can each influence AD pathology in the brain and potentially in regions associated with the brain interface. These findings can shape future translational studies of sex-differences in AD.
INVESTIGATION OF MICROGLIA IN ALZHEIMER'S DISEASE AS PROINFLAMMATORY DRIVERS IN PATIENT DERIVED CELL MODELS (GLIAD)

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Aims: Alzheimer's disease (AD) is the most common cause of dementia, with no curative treatments available, and a traditional focus on neuronal phenotypes. The most common form of AD is sporadic (sAD) with no apparent heritability. sAD is proposed to be triggered by a unique combination of single nucleotide polymorphisms (SNPs) and environmental factors. Due to the fact that neuroinflammation is a common feature of many neurodegenerative disorders and that several SNPs affected in sAD are located in immune-related genes with high expression levels in microglia, we set out to investigate the role of microglia as drivers of AD, exploiting induced pluripotent stem cell (iPSC) technology.

Methods: We have derived iPSCs from both sAD and AD patients (PSEN1 mutations). Subsequently, we evaluated their genetic profile in order to categorize them as either high-risk or low-risk AD lines. iPSCs were differentiated into microglia and neurons to evaluate the inflammatory profile of microglia with different AD polygenic risk score, and their effect on the neural population.

Results: Cytokine profiling revealed clear differences between the groups, with upregulation of proinflammatory cytokines in AD high-risk lines, indicating that microglia profile is dependent on genetic background. Furthermore, AD high-risk microglia have greater detrimental effects on surrounding neurons, observed by increased neuronal cell death and reduced neurite outgrowth in microglia-neuron co-cultures, highlighting the role of microglia in AD progression.

Conclusions: Our in-vitro patient-specific disease models allowed us to recapitulate cell-specific phenotypes, as well as cell-to-cell interactions, making them a great tool for disease modelling and drug target development.
TNF AS A MEDIATOR OF IMMUNE AND METABOLIC ALTERATIONS IN THE 5XFAD MOUSE MODEL OF ALZHEIMER’S DISEASE

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Aims: Objectives: Elevated circulating cytokines are associated with increased risk for cognitive and metabolic impairment. Tumor necrosis factor (TNF) is a driver of immune responses in the periphery and brain, and is implicated in neurodegeneration. We aim to evaluate the role of TNF signaling on the metabolic and immunologic alterations in the brain and periphery that increases the risk for AD.

Methods: Two-month old female 5xFAD mice were fed a high-fat, high-carbohydrate diet (HFHC) or a matched control diet (CD) for 8 weeks. After 1 month, the brain-permeant soluble TNF inhibitor XPro1595 or the brain-impermeant non-selective TNF inhibitor Enbrel were dosed twice weekly for 4 weeks. Plasma, adipose tissue, liver and gut were collected for evaluation of metabolic and immune parameters. Molecular markers of insulin signaling and neuroinflammation were assessed in the hippocampus, hypothalamus and cortex.

Results: HFHC diet increases body weight, gonadal tissue and liver compared to CD groups. HFHC diet promotes a decrease in cecum weight, and small intestine and colon lengths. HFHC elevates circulating insulin, leptin, CCL2 and CXCL2 and decreases plasma IL-2. Enbrel increases liver weight and decreases circulating IL-2 in CD mice. Ongoing behavioral testing and assessments of mRNA and protein expression of insulin signaling and inflammation in the hippocampus, hypothalamus and cortex will determine additional effects of TNF on metabolic and immune impairment in the brain.

Conclusions: Conclusion: Together, these data suggest HFHC diet disrupts peripheral immune and metabolic parameters in the 5xFAD animal model of AD, and create conditions implicated in increased risk for AD development.
REGULATION OF DEPRESSION-LIKE BEHAVIOR VIA FOXP3+ T CELL-MEDIATED IMMUNOMODULATION: A CAUSAL LINK TO ALZHEIMER’S DISEASE PATHOPHYSIOLOGY

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Aims: Alzheimer’s disease (AD) is the most common of dementia among the elderly. Among various causative factors, depression has emerged as a critical factor, increasing the risk of developing AD by 2-4-fold. Thus, elucidating underlying molecular mechanisms linking depression to AD would offer feasible target for preventing AD progression. We have reported the involvement of Forkhead box P3 (FOXP3) modulating the function of regulatory T cells in peripheral immune system, inducing depression-like behavior in mice following stress. However, it is not known if alleviating depression by modulating FOXP3 could reduce the risk of developing AD-like pathologies.

Methods: In the present study, we utilized a FOXP3 or FOXP3x5xFAD mice by treating with diptheria toxin, which causes a reduction of FOXP3 expression. To evaluate anxiety, depression-like behaviors and cognitive impairments, light/dark transition, forced swimming and Y-maze test were performed, respectively. We analysed intestinal immune response using immunohistochemistry and examined molecular changes in hippocampus using RNAseq.

Results: We observed depression, anxiety-like behavior following downregulation of FOXP3. Furthermore, recovery of FOXP3 expression attenuated these behavioral change, accompanied by a significant alteration of Treg/Th17 ratio. To examine the relevance of this mechanism in AD, We are investigating whether reduction of FOXP3 in AD changes intestinal immune response and depletion of peripheral FOXP3 expression alters blood brain barrier permeability leading to neuroinflammation and thereby exacerbating brain cognitive behaviors.

Conclusions: Thus our data suggest FOXP3 as a causal link in the peripheral immune system mediating depression like behavior, leading to exacerbation of AD type brain cognitive deterioration. (Supported by the Altschul Foundation)
Aims: The aim of the current study was to investigate the protective anti-inflammatory effect of Malvidin-O-Glucoside (MG), a natural anthocyanin present in purple grapes, in LPS-induced inflammasome activation in vitro and in a mouse model of Chronic Unpredictable Stress (CUS). This group advocates for the improvement of inflammasome-targeting agents as a potential therapeutic approach to address immune-inflammatory-based disorders.

Methods: Murine primary cortical microglia culture was prepared to examine MG cytotoxicity and pyroptosis. The activation of NLRP3, NLRC4, and AIM2 inflammasomes was induced to study the inhibitory effect of MG. Caspase-1 activity and cytokine levels were explored. MG was administrated at 12.5mg/kg, daily, 15-days prior, and during CUS. Animals were subjected to two stressors per day for 28 days. Depressive and anxiety-like behavior was monitored using elevated plus maze, sucrose preference test, or forced swim test. MG bioavailability was measured by pharmacokinetics.

Results: MG treatment did not exhibit cytotoxicity in vitro. After LPS-induced inflammation in vitro, MG was able to inhibit NLRC4, NLRP3, and AIM2 inflammasomes, showing its anti-pyroptotic function. Caspase-1 activity was also reduced. After CUS, animals treated showed IL-1β gene downregulation and a decrease in protein levels in the hippocampus. Behavioral studies reported a decrease in anxiety- and depressive-related behavior. Pharmacokinetic studies reveal the presence of two metabolites Malvidin-Glucuronide and Malvidin-Glucoside in both brain and plasma.

Conclusions: This study suggests that the polyphenol MG presents anti-inflammatory potential after LPS-inflammation induction in vitro and in a model of CUS, rendering a putative therapeutic profile to address stress-induced behavioral impairment. Acknowledgments: Supported by the Altschul Foundation
NEUROPROTECTIVE EFFECTS OF ASTAXANTHIN IN PRECLINICAL MODELS OF ALZHEIMER’S DISEASE

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Aims: Alzheimer’s disease (AD) is the most common cause of dementia, and it accounts for about 70\% of all dementia cases. The pathological manifestations of Alzheimer’s disease include aggregation of misfolded protein fragment beta-amyloid outside neurons called plaques, hyper-phosphorylation of protein tau inside neurons called tangles, neuronal loss, synaptic degeneration, and neuroinflammation. Until date, there are no disease-modifying therapies available. Astaxanthin, a lipid-soluble xanthophyll beta-carotenoid synthesized by many microorganisms, has been reported to exhibit anti-inflammatory and neuroprotective functions.

Methods: To investigate the neuroprotective effects of astaxanthin, primary mouse hippocampal neurons and organotypic brain slice cultures were treated with either amyloid beta (Aβ\textsubscript{1-42}) or lipopolysaccharides (LPS) and co-incubated with astaxanthin. Expression levels of relevant markers were examined at protein and mRNA levels by quantitative real time polymerase chain reaction (qRT-PCR), immunofluorescence and immunoblotting techniques. Cytokine release was determined using an immunosorbent assay (Mesoscale discovery, MSD).

Results: Treatment of LPS-stimulated brain slices with astaxanthin significantly reduced the secretion of pro-inflammatory cytokines (TNF-α, IL-6, KC/GRO) into the supernatant. In addition, we observed prevention of both neuronal and synaptic loss as measured by an increased relative signal intensity of neuronal marker NeUN, and post-synaptic marker PSD95 in primary mouse hippocampal neurons treated with Aβ\textsubscript{1-42} and co-incubated with astaxanthin.

Conclusions: Our data suggest that astaxanthin may be a therapeutic candidate by ameliorating pathophysiological manifestations associated with Alzheimer’s disease.
Aims: Dementia is a significant public health problem which leads to poor health outcomes. Inflammatory biomarkers have been found to be important in signaling cardiovascular disease. In this study, we determined whether the longitudinal evidence supports the need to use biomarkers like C-reactive protein (CRP) as poor prognostic indicators for cognitive decline, especially in individuals with congestive heart failure (CHF).

Methods: We used population-based cohort study of 1999-2002 National Health and Nutrition Examination Surveys with mortality data obtained through 2015. Adults aged 60 years or older with CHF were assessed for cognitive skills using Digit Symbol Substitution Test (DSST). Outcomes of all-cause mortality were evaluated using Cox regression at various levels of CRP (vs. ≤2ug/dL).

Results: Percent of deaths from low cognitive function among the population (N=160) were higher among Hispanic Americans (12.0%) than Caucasians (9.4%). The mean follow-up was 8.2 years. For all-cause mortality, the overall unadjusted hazard ratio (HR) of low cognitive function was 1.91 (95% confidence interval [CI], 1.05-3.45, p < 0.03). Adjusted HR was elevated, 15.31 (CI 4.67-50.15, p < 0.001), among elevated CRP and low cognitive function but closer to 1.0 (1.36 CI 0.74-2.51, p < 0.31) among those with normal CRP and low cognitive function, after controlling for medical (obesity) and demographic risk factors (age and sex).

Conclusions: Our research shows that low cognitive function leads to higher mortality, especially among individuals with elevated biomarkers once they have developed CHF. Improved identification of dementia, increased surveillance efforts, and addressing issues with health equity are needed to improve survival.
PROTECTIVE EFFECT OF CROCIN ON LIPOPOLYSACCHARIDE-INDUCED NEUROINFLAMMATION IN A RAT MODEL OF PARKINSON’S DISEASE IS ASSOCIATED WITH DECREASED EXPRESSION OF AIM2 INFLAMMASOME

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Aims: Mechanisms associated with activation of inflammasome complex and loss of dopaminergic neurons due to chronic neuroinflammation is not entirely defined. Therefore, this study was conducted to evaluate the effect of crocin on the inflammasome complex in the experimental model of Parkinson disease (PD).

Methods: PD was induced by stereotaxic injection of lipopolysaccharide (LPS). Animals received crocin one week before lesion intraperitoneally and continued for 21 days. Open field (OF) and elevated plus maze test were used as behavioral assays. Inflammatory cell infiltration was assessed through hematoxylin and eosin (H & E). In addition, immunostaining, immunoblotting and Real-time PCR were used to evaluated compartment are involed in PD.

Results: Time spent in center for OF test was diminished in LPS group, while treatment with 30 mg/Kg of crocin significantly increased this time. H &E staining showed significant increase of cell infiltration at LPS injection site, while crocin reduced it. Few caspase-1 and IL-1β positive cells were observed in crocin treated animals, whereas number of positive cells was increased in LPS group. Significant decrease of TH expression was confirmed in LPS group, whereas crocin significantly increased it. The expression of IL-1β, IL-18, and AIM2 genes in LPS group was significantly increased. On the other hand, 30 mg/Kg of crocin was able to significantly reduce expression levels of these genes along with NLRP1.

Conclusions: Our results suggest that crocin can improve neuroinflammation in PD by decreasing IL-1β and caspase-1 possibly via inhibition of AIM2 and NLRP1 inflammasomes, introducing promising targets for treatment of PD in the future.
Aims: Alzheimer's disease (AD) is an age-related neurodegenerative disorder affecting around 35 million individuals worldwide. Besides aging, various comorbid factors increase the risk of AD, including air pollution and asthma. Epidemiological studies have reported a 2.17-fold higher risk of dementia in asthmatic patients. However, the molecular mechanism(s) underlying this asthma-associated AD exacerbation is not known. This study was designed to explore house dust mite-induced asthma effects on AD-related brain changes using the AppNL-GF transgenic mouse model.

Methods: Male and female C57BL/6 wild type and AppNL-GF mice (8-9 months old) were exposed to either saline or house dust mite (dose: 833μg/kg in saline) every alternate day for 16 weeks. Mice were sacrificed at the end of the experiment, and broncho-alveolar lavage fluid (BALF), lungs, and brains were collected. BALF was analyzed for immune cell markers and inflammatory mediators. In addition, Alcian blue and Masson's trichrome staining was performed on lung sections while brain sections were immunostained for Aβ. Finally, frozen hippocampi were used to perform Aβ ELISAs.

Results: As expected, the asthma-induced group showed increased pulmonary inflammatory cells, cytokine levels, and mucus and collagen production. Interestingly, hippocampi from asthma-induced mice had elevated Aβ plaque load and increased levels of soluble Aβ 1-40/42 and insoluble Aβ 1-40.

Conclusions: Based on our study, increasing cases of both diseases, and their comorbidity with age, understanding a mechanistic relationship tying progression of AD and asthma may provide a novel therapeutic intervention for both chronic diseases.
Aims: To determine whether the activation of monocytes can be used as a biomarker for the progression of Alzheimer's disease (AD).

Methods: We separated form healthy, mild cognitive impairment (MCI) and Ad patients their monocytes. We determined by FACScan the phenotypes of these monocytes, their functionality and their capacity to differentiate in macrophages.

Results: We found that monocytes were already activated at the MCI stage mainly regarding the free radical production. In the mean time there was a shift in the phenotypes of monocytes toward the non-classical subsets. The differentiation was also shifted to M2 phenotype.

Conclusions: We conclude that monocytes as they are activated already as in the MCI stage of the disease may serve as biomarkers and could represent a good target for treatment options.
A COMPARATIVE PHARMACOLOGICAL MODEL OF ALZHEIMER DISEASE IN RAT

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Aims: The fact that Alzheimer’s disease (AD) is until now an incurable pathology necessitated and still requires the development of new animal models. This contributed greatly to the diversity and abundance of animal models of AD proposed in the literature. But that does not add a point to the effort needed to complete our understanding of the complex and overlapping mechanisms of this pathology. In this context, this work aims to do a comparative study between different pharmacological animal models based on the most reported theories to explain the pathology.

Methods: Female Wistar rats (5-months old) were administered intracebroventricularly by artificial cerebrospinal fluid aCSF, beta amyloid BA1-42, okadaic acid, lipopolysaccharides (LPS), buthionine sulfoximine or by a mixture of these different molecules. Cognitive performance was assessed one week or one month after stereotaxic surgery.

Results: Our results, show that only the BA1-42 and the mixture models induced a persistence deficit in the working memory, recognition memory and spatial memory in rats. As the hippocampus (Hip) is particularly involved in memory behavior, we analyzed long-term neuroadaptations in the Hip using spectrophotometric and histological methods to assess oxidative stress changes and neuronal loss, respectively. We found that the behavioral impairments were accompanied by irreversibly oxidative stress marked changes and neurodegenerescence in the Hip.

Conclusions: This study provides promising data on the modeling of AD in order to develop an effective therapeutic approach.
AGE-RELATED NEUROINFLAMMATION AND PATHOLOGY IN THE LOCUS COERULEUS AND HIPPOCAMPUS: BETA-ADRENERGIC ANTAGONISTS EXACERBATE IMPAIRMENT OF LEARNING AND MEMORY IN AGED MICE

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Aims: The locus coeruleus (LC) is the main source of noradrenergic input to the forebrain and hippocampus, and may be vulnerable to degeneration and contribute to age-related cognitive decline and neuroinflammation. This study was designed to test the effects of age and the acute effects of beta-adrenergic agonist and antagonist on pathology in the LC and hippocampus.

Methods: Behavioral Testing, Immunohistochemistry, Western Blot, Multiplex mouse cytokine assay, Proteomics

Results: An increase in hippocampal and LC microgliosis and inflammatory proteins in the hippocampus was detected in aged mice. We report pathological hyperphosphorylation of the postsynaptic NMDA receptor subunit 2B (NR2B) in the hippocampus, suggesting neuronal hyperexcitability. Furthermore, the aged proteome revealed an induction in proteins related to energy metabolism, and mitochondria dysfunction in the LC and hippocampus. In a series of hippocampal dependent behavioral assessment tasks, acute beta-adrenergic agonist or beta blocker administration altered learning and memory behavior in both aged and young mice.

Conclusions: In summary, our data provide evidence for age-related neuroinflammation in the locus coeruleus and hippocampus alongside evidence for metabolic challenge in the locus coeruleus and extrasynaptic deficiency of NMDA receptor signaling in the hippocampus. This cognitive impairment in aged mice is exacerbated with acute beta-blocker delivery. Further studies are needed to understand mechanisms linking metabolic stress, neuroinflammatory signaling from astrocytes and microglia, and the extra-synaptic pathology. Future studies will be needed to explore the impact of long-term exposure to beta-blockers on neuroinflammation, brain pathology, and behavioral function.
Aims: In Lewy bodies Dementia (DLB) and Alzheimer's disease (AD) cortical cholinergic neurotransmission is compromised, and this is thought to contribute to cognitive decline and the neuropsychiatric symptoms experienced by these patients. Compelling evidence has shown that the peripheral immune system plays an important role in neuroinflammatory diseases and, new successful therapeutic strategy may need to target the crosstalk between the nervous and immune systems. The aim of our study was the comparison of muscarinic cholinergic receptor (CHRM) subtypes, M1 and M4, mRNA expression in PBMCs from healthy, AD and DLB subjects.

Methods: CHRMs expression in PBMC of DLB and AD compared to healthy controls (HC) were assayed by the quantitative Real-Time PCR. The relative fold changes in gene expression were determined by the $2^{-\Delta\Delta CT}$ method.

Results: Although preliminary, our findings showed that peripheral CHRM1 expression was higher and CHRM4 was lower in DLB and AD compared to HC, whereas both CHRM1 and CHRM4 levels were higher in AD compared to DLB patients. ROC curve analysis showed the diagnostic performances of each receptor gene expression in discriminating each patient group from HC and DLB from AD patients.

Conclusions: In view of the easy accessibility of PBMC for research and diagnostic purposes, assay of muscarinic cholinergic receptors in PBMC, mirroring what happen in the brain, may contribute to assess cholinergic dysfunction in neuroinflammatory/neurodegenerative status.
Aims: Neuroimmune responses from astrocytes and microglia play a key role in the pathogenesis of neurodegenerative diseases. Identifying the key mechanisms underlying neuroimmune signaling in large datasets is key to finding new therapeutic targets. Our objective was to build causal biological network (CBN) models to interpret large datasets.

Methods: CBN models assemble mechanistic biological knowledge from the scientific literature in a structured computable format that facilitates mechanistic interpretation. CBNs capture molecular nodes connected with edges, which indicate the causal relationship between these nodes. For a subset of nodes, the mRNAs regulated by these nodes are known. By comparing these known regulated mRNAs with all the mRNA changes seen in an experimental dataset, individual molecular drivers are inferred. We applied CBN models of astrogliosis and microgliosis to a neuroinflammation transcriptomic dataset. We assessed the GSE75246 dataset—which contains RNA sequencing data from astrocytes and microglia isolated from lipopolysaccharide (LPS)-treated mice—and ranked the nodes according to their inferred significance values.

Results: The analysis highlighted several molecular drivers that are important in both microglial and astrocyte samples, including inflammatory markers such as interferon family members (Ifna2, Ifna4, Ifnar1, Ifng, and Ifnb1), interleukin-6 family members (Il6, Osm, and Lif), and Toll-like receptors (Tlr2, Tlr3, Tlr4, Tlr5, and Tlr9). Key drivers unique to the microglia included Gab2, Ripk1, and the Notch family, while drivers unique to the astrocytes included the dopamine D2 receptor family and Cxcl12.

Conclusions: CBN models are useful tools for identifying general and cell-type-specific molecular drivers in transcriptomic datasets.
Aims: The translocator protein 18kDa (TSPO) is a conserved outer mitochondrial membrane protein, implicated in inflammation, cell survival and proliferation. In the central nervous system, the expression of TSPO being markedly upregulated in M1 pro-inflammatory microglia during various disease states such as Alzheimer’s disease. Synthetic as well as endogenous ligands with agonistic or antagonistic properties can modulate the function of TSPO. Thus, TSPO ligands can act as putative therapeutic agents during neuroinflammatory processes. In the present study, we examined the efficacy of a new TSPO ligand, named TEMNAP, in mitigating inflammatory process compared to the classic TSPO ligand, PK-11195.

Methods: Lipopolysaccharide (LPS)-activated microglial cells were used as an in vitro model to study the anti-inflammatory effects of TEMNAP. In particular, we explored the efficacy of nanoparticle-mediated delivery of TEMNAP by investigating the molecular and the morphological properties of LPS-stimulated BV2 microglia.

Results: We demonstrated that the exposure of BV2 microglia with TEMNAP significantly reduced the LPS-induced microglia proliferation and strongly prevented the expression of the pro-inflammatory marker iNOS, as well as, the production of nitric oxide in LPS-stimulated microglia. In addition, we found that nanoparticle-mediated delivery of TEMNAP significantly prevented the LPS-induced proliferation of hyperactivated microglia and strongly reduced the upregulation of the M1 pro-inflammatory markers iNOS and CD86. Accordingly to our in vitro observations, preliminary results in vivo demonstrated the anti-inflammatory efficacy of the newly synthesized TSPO ligand in the heterozygous male Tg2576 mice, an in vivo model of Alzheimer’s disease.

Conclusions: Collectively, our results revealed a strong anti-inflammatory efficacy of the new compound TEMNAP.
Aims: Despite well-documented maladaptive neuroinflammation in Alzheimer’s disease (AD), the principal signal that drives memory and cognitive impairment remains elusive. Type I interferon (IFN) is an innate immune cytokine aberrantly elicited by β amyloid plaques. Here, we seek to understand its role in cognition and neuropathology relevant to AD.

Methods: We introduced a genetically-encoded iIFN-responsive reporter system into 5XFAD mice, a β-amyloid plaque model, to gauge IFN-stimulated cellular response. We administered an antibody that specifically blocks the signaling of IFN receptor into 5XFAD mice and examined their memory performance, neuropathologies, and gene expression. Moreover, we generated and analyzed 5XFAD mice that lack IFN receptor selectively in microglia or neural cells.

Results: Here, we reveal an age-dependent, brain-wide, and profound accrual of brain cells responding to IFN signaling activation in 5XFAD mice. Long-term blockade of IFN receptor rescued both memory and synaptic deficits, and also resulted in reduced microgliosis, inflammation, and neuritic pathology. Interestingly, microglia-specific IFN receptor ablation attenuated the loss of post-synaptic terminals, whereas IFN signaling in neural cells contributed to pre-synaptic alteration and plaque accumulation. Intriguingly, IFN pathway activation displayed a strong inverse correlation with cognitive performance, promoting selective synapse engulfment by microglia rather than amyloid plaques.

Conclusions: IFN signaling represents a critical module within the neuroinflammatory network of AD and prompts a concerted cellular state that is detrimental to memory and cognition.
SHIP1 REGULATES TREM2 SIGNALLING IN HUMAN IPSC MACROPHAGES

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**Aims:** TREM2 signalling regulates microglial function, and activation of the TREM2 cascade may mediate a protective phenotype. TREM2 mutations and variants of signalling molecules in the TREM2 cascade confer Alzheimer’s disease (AD) risk or protection. One of these risk genes, INPP5D (SHIP1) is believed to inhibit TREM2 signalling by opposing SYK phosphorylation to prevent over-stimulation of the pathway, and a risk associated AD variant of SHIP1 has been identified which increases SHIP1 expression. We aimed to investigate the interaction between SHIP1 and TREM2 in human iPSC macrophages, to confirm whether SHIP1 inhibition may enhance TREM2 signalling.

**Methods:** TREM2 / SHIP1 crosstalk was explored using isogenic WT and SHIP1 KO human iPSC macrophages. Western blotting was used to assess the expression of TREM2. An AlphaLISA was developed to determine the levels of soluble TREM2 in each line. The effect of SHIP1 inhibition on TREM2 signalling was investigated using specific SHIP1 inhibitors, and read-outs included assessment of SYK phosphorylation and intracellular Ca\textsuperscript{2+} flux. Finally, we explored the effect of SHIP1 inhibition on macrophage phagocytosis.

**Results:** SHIP1 KO iPSC macrophages showed reduced TREM2 expression and greater TREM2 shedding when compared to WT iPSC macrophages. SHIP1 inhibition was found to potentiate TREM2 mediated SYK phosphorylation and intracellular Ca\textsuperscript{2+} flux. Furthermore, TREM2 mediated phagocytosis was enhanced by SHIP1 inhibition.

**Conclusions:** Our data indicates the presence of a SHIP1 mediated negative feedback loop that regulates TREM2 expression, signalling and phagocytosis. SHIP1 inhibition may therefore represent a novel therapeutic approach for the treatment of Alzheimer’s disease by enhancing protective microglial functions.
Aims: Neuroinflammation in patients with Alzheimer’s disease (AD) and related mouse models has been recognized for decades, but the contribution of the recently described meningeal immune population to AD pathogenesis remains to be addressed.

Methods: Here, using the 3xTg-AD model, we report an accumulation of interleukin-17 (IL-17)-producing cells, mostly gd T cells, in the brain and the meninges of female, but not male, mice, concomitant with the onset of cognitive decline.

Results: Critically, IL-17 neutralization into the ventricles is sufficient to prevent short-term memory and synaptic plasticity deficits at early stages of disease. These effects precede blood-brain barrier disruption and amyloid-beta or tau pathology, implying an early involvement of IL-17 in AD pathology. When IL-17 is neutralized at later stages of disease, the onset of short-memory deficits and amyloidosis-related splenomegaly is delayed.

Conclusions: Altogether, our data support the idea that cognition relies on a finely regulated balance of “inflammatory” cytokines derived from the meningeal immune system.
Aims: Synaptic loss is one of the earliest pathological hallmarks and the strongest marker of cognitive decline in Alzheimer's disease (AD). A strong genetic predisposition is linked to AD, and genome-wide association studies have pointed out hundreds of genes associated with the risk of developing the disease. With this background, our objective is to develop a cell-based screen to assess the impact of each genetic risk factor on synaptic density.

Methods: We screened a lentiviral shRNA library targeting 200 AD genetic risk factors using primary rat hippocampal neurons in 384-wells plates. Immunofluorescence was performed to reveal pre- and post-synaptic compartments and the neuronal network. Synaptic density was then assessed through high content analysis by assigning each post-synaptic structure to the nearest pre-synaptic structure using Columbus and Matlab software.

Results: We identified 14 shRNAs targeting AD risk genes which positively (Log2>1) or negatively (Log2<-1) impacted synaptic density in our model. These results could help us (i) to prioritize genes of interest in complex GWAS Loci and (ii) to point out a role for some unexpected AD genetic risk factors in synaptic function.

Conclusions: We have developed a high-content screening approach which allows us to define the genetic risk factors that are susceptible to be involved in synaptic dysregulation observed in the early stages of AD.
A MOUSE BRAIN PROTEOME STUDY TO IDENTIFY NOVEL PROTEIN TARGETS TO MODULATE BRAIN HYPEREXCITABILITY IN DEMENTIA

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Aims: We identified loss of function mutations in dipeptidyl peptidase 6 (DPP6) in patients with Alzheimer's disease (AD) or frontotemporal dementia (FTD), Cacace et al., 2019. DPP6 is expressed in neurons in a multimeric protein complex with the potassium channel K\textsubscript{v}4.2, regulating gating properties and surface expression of K\textsubscript{v}4.2 in brain. Loss of DPP6 is known to cause dendritic hyperexcitability of neurons in DPP6-KO mice (Sun et al., 2011), but knowledge on the consequences of DPP6 loss on a whole brain proteome is lacking.

Methods: Label free liquid chromatography tandem mass spectrometry (LC-MS/MS) performed on brain tissue of Dpp6 wild-type (Dpp6\textsuperscript{WT}, n=12), heterozygous knock-out (KO; Dpp6\textsuperscript{+/-}, n=12) and Dpp6 full KO (Dpp6\textsuperscript{-/-}, n=11) mice. MaxQuant algorithm used for data analysis. Stringent data quality filter and differential expression analysis performed with Perseus (1.6.2) and enrichment analysis with Gene Ontology (GO).

Results: 2662 protein groups were reliably quantified. Differential expression analysis retained n=37 significant proteins. Hierarchical clustering and functional analysis identified 2 clusters. In the first cluster 4 proteins downregulated in Dpp6\textsuperscript{+/-} and Dpp6\textsuperscript{-/-} mice, including Dpp10, similar function as Dpp6. In the second, 32 proteins upregulated in Dpp6\textsuperscript{+/-} and Dpp6\textsuperscript{-/-} mice. The latter enriched in proteins involved in cellular ion homeostasis and negative regulation of gene silencing.

Conclusions: Validation will be done in mouse brain tissue and in a DPP6 KO cellular model of human iPSC derived neurons. A better understanding of the alterations in brain when DPP6 is lacking will provide different targets to possibly compensate the effect of DPP6 loss.
ASSOCIATIONS BETWEEN MARKERS OF SYNAPTIC DYSFUNCTION AND TAU ACCUMULATION, GLIAL ACTIVATION, AND NEURODEGENERATION IN ALZHEIMER’S DISEASE

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Aims: While Alzheimer’s disease (AD) is characterized by β-amyloid and tau, growing evidence suggests that synaptic dysfunction is a key feature of AD and may be a mechanism linking these pathologies to subsequent cognitive decline. The current aim was to investigate how levels of synapse proteins selected from a novel panel of synaptic biomarkers in cerebrospinal fluid (CSF) are associated with tau accumulation, glial activation, and neurodegeneration in an AD-enriched cohort.

Methods: Participants were selected from the TRIAD cohort (N=105; 20 young, 44 cognitively normal, 41 cognitively impaired). Mass spectrometry methods were used to quantify pre- and post-synaptic proteins in CSF, including 14-3-3 zeta/delta, gamma-synuclein, neurogranin, and syntaxin and pentraxin proteins (Nilsson, 2021;10.1002/dad2.12179). Group comparisons and regression models with CSF levels of tau and glial activation markers were conducted, along with mediation analyses and correlations with AD-signature cortical thickness.

Results: Of 12 quantified synaptic proteins, nine showed significant age effect and ten showed a significant difference in β-amyloid+/tau+ compared to β-amyloid-/tau-. Regressions with CSF p-tau231 and p-tau217 were significant for all synaptic proteins, as were regressions with CSF YKL-40 and GFAP. Mediation analysis with tau (predictor) and glial activation (mediator) showed that seven synaptic proteins’ associations with tau were significantly mediated by glial activation. These seven proteins matched those that were significantly correlated with AD-signature cortical thickness, with the exception of PEPB-1.
Conclusions: Our study of CSF synaptic dysfunction biomarkers in AD suggests that synaptic proteins whose relationship with tau is mediated by glial activation may be more closely associated with reduced cortical thickness.
Aims: In Alzheimer’s disease (AD), cognitive decline correlates with synapse dysfunction and loss. Increasing evidence suggests that deregulation of the Wnt pathway contributes to synapse vulnerability in AD. Recent studies showed that the secreted Wnt antagonist Dkk3 is increased in plasma and CSF of AD patients and accumulates in amyloid-β (Aβ) plaques. However, Dkk3 function in the brain remains unexplored. Here, we investigated the contribution of Dkk3 to AD pathogenesis.

Methods: Human brain samples, J20 and NLGF mouse lines were used. Dkk3 levels and localisation were analysed by western blot and confocal microscopy. In vivo Dkk3 loss-of-function was performed by stereotactic injection of adeno-associated viruses expressing shRNAs. Synapse number and function were evaluated by confocal microscopy and patch-clamp recordings. Behaviour was assessed using several paradigms such as Morris-water-maze.

Results: Our results show that Dkk3 secretion is increased before plaque formation and accumulates at Aβ plaques in AD mouse models. DKK3 levels are also increased in the hippocampus of AD patients. Analyses of RNAseq data reveal that increased DKK3 expression is associated with AD. Importantly, our gene and SNP-based analyses demonstrated a genetic link between DKK3 and AD. Gain-of-function experiments show that Dkk3 decreases excitatory synapse number but increases inhibitory synapses. Importantly, in vivo Dkk3 loss-of-function ameliorates synaptic changes in the hippocampus at early and late stages and improves memory in J20 mice.

Conclusions: Our genetic and functional studies demonstrate a novel role for Dkk3 in AD. We propose Dkk3 as a therapeutic target to reduce synapse dysfunction and cognitive deficits in AD.
A GENETIC VARIANT OF THE WNT CO-RECEPTOR LRP6 LINKED TO LOAD PROMOTES SYNAPSE IMPAIRMENT THE AGEING AND ALZHEIMER’S DISEASE BRAIN

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Aims: Synapse loss is the strongest correlate to cognitive decline in Alzheimer’s disease (AD). Substantial evidence exists for the role of canonical Wnt signalling, through the co-receptor LRP6, in synapse integrity in the mature brain. Importantly, deficient Wnt signalling has been suggested to contribute to synapse loss in AD. A variant of LRP6 (LRP6-Val), with reduced Wnt signalling, is linked to late onset AD (LOAD). However, the in vivo effect of LRP6-Val on synaptic connectivity in the ageing brain and in AD has not been addressed.

Methods: We generated a novel knock-in mouse model carrying the LRP6-Val variant. Homozygous mice develop normally and show no obvious morphological abnormalities in the adult brain. We examined LRP6-Val homozygous mice for possible changes in synapse number using confocal microscopy at different ages. LRP6-Val mice were crossed to the AD KI model, NL-G-F, to examine the contribution of this variant to AD pathogenesis.

Results: We showed that mice carrying the LRP6-Val variant exhibit synapse loss in an age-dependent manner (from 12 to 16 months). In contrast, LRP6-Val; NL-G-F mice exhibit a significant loss of synapses around plaques at early stages (7 months). However, no differences in plaque load were observed.

Conclusions: Our work highlights the importance of Wnt-LRP6 signalling in synapse integrity in the ageing brain and uncover, for the first time, that carrying LRP6-Val confers progressive synaptic defects. Our studies uncover a novel role for the LRP6 variant in synaptic vulnerability during ageing and in the context of AD.
RELEASE OF SYNAPTIC AD BIOMARKERS UPON AMYLOID BETA EXPOSURE OF HUMAN IPSC DERIVED CORTICAL NEURONS

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Aims: The Alzheimers disease (AD) diagnostic field has leaped forward, with novel blood biomarkers emerging. However, there is still a need for novel biomarkers enabling early diagnosis and monitoring of disease progression. As synaptic degeneration is an early event in AD pathology, synaptic proteins are potential AD biomarkers. Understanding the mechanisms behind synaptic biomarker release is therefore important if they are to be used in the clinic. The aim of this study was therefore to investigate if the release of synaptic biomarker proteins is dependent on oligomeric Aβ exposure.

Methods: Human iPSCs were differentiated into cortical neurons following a well characterized protocol. 100 μM of synthetic Aβ42 was oligomerized in phenol-red free media at 4°C for 24h. Mature neurons were incubated with 500 nM or 1 μM oAβ for 48 hours (or 1 μM scrambled control). Intracellular protein expression was analyzed using western blot. Secreted synaptic proteins were analyzed using ELISA or mass spectrometry.

Results: We found that intracellular levels of pre-synaptic proteins, including SNAP25 and vGlut1, decreased upon oAβ42 treatment, whereas post-synaptic proteins such as PSD95 and NGRN did not change. Preliminary data show that oAβ42 exposure increased secretion of Tau, Neurogranin and Rab GDI alpha, whereas SNAP25 decreased. Other synaptic proteins were unchanged.

Conclusions: In conclusion, iPSC derived neurons is a good in vitro model to study the biology behind synaptic biomarker release. oAβ42 treatment did not affect all synaptic proteins in a similar manner, and it is important to further study mechanisms behind the release of specific synaptic proteins.
THE SYNAPTIC MARKER CYCLASE-ASSOCIATED PROTEIN (CAP2) IS INCREASED AND CORRELATES WITH TAU PATHOLOGY IN ALZHEIMER’S DISEASE

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Aims: The Cyclase-associated protein 2 (CAP2) dimerization is known to play a role in synaptic plasticity in AD models but has never extensively tested in vivo in cerebrospinal fluid of patients. Aim of the study was to evaluate the levels of CAP2 in cerebrospinal fluid of AD and other neurological disorders and their correlation with Abeta and tau biomarkers in vivo.

Methods: a preliminary set of 53 AD patients (mean age 69.3 ± 7.5 years) and 21 healthy controls underwent CSF analyses for Tau, P-tau and Abeta amyloid and an extensive cognitive, behavioral and motor assessment. CAP2 levels were assessed using standard CSF ELISA. Correlations between CSF biomarkers were evaluated using partial correlation adjusted for the effect of age, sex and disease duration.

Results: AD patients exhibited higher CAP2 levels compared to controls (22.4 ± 8.6 vs 16.1 ± 7.3) adjusting for the effect of age and sex. Partial correlation CSF analyses showed a positive correlation between CAP2 levels and Tau (r=0.48, p=0.001), P-tau (r=0.46, p=0.001) and P-tau/Amyloid ratio (r=0.28, p=0.003).

Conclusions: these preliminary findings indicate that CAP2 levels are increased in AD patients and correlated with tau pathology in Alzheimer’s disease patients. Further ongoing longitudinal studies including other synaptic dysfunction markers are warranted in order to extend these preliminary findings.
DYSREGULATION OF ASTROCYTE CALCIUM SIGNALING AND GLIOTRANSMITTER RELEASE BY A53T MUTANT HUMAN ALPHA-SYNUCLEIN

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Aims: Previous work has demonstrated the altered homeostatic function of astrocytes in α-synucleinopathies but the role of the bidirectional astrocyte-neuronal communication in these diseases remains poorly understood. We tested the hypothesis that selective α-synuclein accumulation in astrocytes induces Ca²⁺ dynamics dysregulation and anomalous glutamate release from astrocytes.

Methods: Males and females 1-6-month-old transgenic mice expressing human wild type (WT), A53T mutant, and A30P mutant α-synuclein under the control of mouse Prp or CamKII-promoter were used in this study. Astrocyte Ca²⁺ activity was monitored with confocal imaging. CA1 neurons of the hippocampus were recorded using the whole-cell patch-clamp technique.

Results: We found that expression of A53T mutant but not WT or A30P α-synuclein markedly enhanced the intrinsic Ca²⁺ activity and gliotransmitter release (as monitored by the presence of slow inward currents or SICs) from astrocytes in the CA1 area of the hippocampus. We also recorded astrocyte Ca²⁺ signals and SICs in slices obtained from a transgenic mouse line that specifically overexpresses A53T mutant α-synuclein in neurons (CamKII-A53T line). We found no difference in astrocytic Ca²⁺ event frequency or SICs frequency in these mice. However, mEPSC amplitude and frequency were still reduced in these mice.

Conclusions: These data indicate that, in astrocytes, both intracellular Ca²⁺ events and gliotransmitter release from astrocytes are increased in the A53T mutant α-synuclein transgenic mouse model. However, these changes were not present in mice expressing A53T-mutant α-synuclein exclusively in neurons. These results indicate a cell-autonomous effect of pathogenic α-synuclein A53T in astrocytes that may underlie the altered neuronal function observed in α-synucleinopathies.
Aims: Mutations of the gene encoding Presenilin 1 (PS1) are responsible for most cases of familial Alzheimer disease (FAD), which shares clinical and neuropathological phenotypes with the more common sporadic AD (SAD). It is now evident that disruption in synaptic activity and loss of synapses are the earliest events in AD preceding the clinical manifestation of the disease. The aim of our study is to identify the underlying mechanisms of NMDAR synaptic dysfunction, which may causally precipitate cognitive deterioration in the absence of amyloid neuropathology in a mouse model of FAD.

Methods: We used two independently constructed PS1 FAD knock-in (KI) mouse models, one expressing PS1 M146V, and the other expressing I213T. We also utilized postmortem human brain tissue from non-demented carrying WT PS1 and FAD heterozygous for mutant PS1 S170F. Biochemical fractionation was performed to examine synaptic localization of proteins, while electrophysiology experiments were conducted to measure NMDAR synaptic functions.

Results: We have reported alteration of NMDAR-mediated excitatory postsynaptic currents (EPSCs) at CA1 synapses by PS1FAD mutants. Here, we have shown that PS1FAD mutants significantly increased synaptic localization of the mutant protein in mouse brains without affecting the expression of the mutant protein. Postmortem human brains, albeit carrying different PS1FAD mutation, also exhibited aberrant membrane PS1 localization. Our results suggest that synaptic mislocalization of mutant PS1 may underlie NMDAR synaptic dysfunction in FAD.

Conclusions: Together, our findings suggest that aberrant synaptic localization of PS1 may have relevance to human FAD, and that manipulating PS1 membrane trafficking might be a viable therapeutic approach to the disease.
POSTERS

SYNAPTIC DYSFUNCTION AND PLASMA NFL CHANGES IN RTG4510 MOUSE MODEL OF ALZHEIMER’S DISEASE

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Aims: The rTg4510 mouse, a model of tauopathy, overexpresses P301L mutant human Tau in the forebrain. This line of mice exhibits age-dependent synaptic dysfunction resulting from region-specific progression of neuropathology and associated cognitive decline. We studied the progression of electrophysiological deficits and changes in plasma levels of neurofilament light chain (NFL) protein, a biomarker for neurodegeneration, to monitor disease progression and to provide a physiological endpoint and biomarker for efficacy studies.

Methods: We used extracellular field potential recordings in hippocampal slices to study short-term and long-term plasticity (LTP) at the Schaffer collateral-CA1 pyramidal cell synapses in 2-3 month and 6-7 month-old rTg4510 mice. Plasma samples were assayed using the Simoa™NF-light®Advantage Kit (Quanterix).

Results: Six-month-old rTg4510 mice exhibit a correlated reduction in pre-synaptic fiber volley (FV) amplitude (~50%) and field excitatory post-synaptic potential (fEPSP) slope (~40%) compared to wildtypes (WT); the effect was not seen in 2-month-old mice. Basal synaptic transmission is not altered, since fEPSP slope, controlled for FV amplitude, remained unchanged. Both paired-pulse facilitation (PPF) and LTP-induced with high-frequency stimulation, were reduced in 6-month-old rTg4510 mice. Reduction of PPF suggests a deficit occurring in pre-synaptic residual neurons. Consistent with the electrophysiological findings, NFL protein levels were elevated in plasma starting at 4 months of age. This genotypic increase was prevented by repressing mutant Tau expression through doxycycline treatment.

Conclusions: NFL levels and electrophysiology, in parallel with other behavioral, biochemical, immunohistochemical read outs, can be used to measure the efficacy of a putative treatment in this preclinical model of tauopathy.
GLOBAL EXCITATORY TO INHIBITORY SYNAPTIC IMBALANCE IN HIPPOCAMPUS AND TEMPORAL CORTEX IN MILD COGNITIVE IMPAIRMENT AND ALZHEIMER’S DISEASE

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Aims: Individuals at distinct stages of Alzheimer’s disease (AD) show abnormal electroencephalographic activity which has been linked to network hyperexcitability and cognitive decline. Using electrophysiology and proteomics of human synapses from the hippocampus and temporal cortex of control, mild cognitive impaired (MCI) and AD individuals we aim to determine the global synaptic balance in hippocampus and temporal cortex at distinct stages of neuropathology.

Methods: Electrophysiological synaptic E/I ratios in post-mortem samples from the temporal cortex of individuals with MCI (n = 6) or AD (n = 6) compared to non-demented controls (n = 6), and the hippocampus (MCI, n = 8; AD n = 11, CTRL = 8) were assessed by microtransplantation of synaptic membranes (MSM). Proteomics of synaptosomes from temporal cortex were analyzed in the context of their electrophysiological responses.

Results: We found important relationships between the amplitude of ion currents and Mini Mental State Examination (MMSE) scores. In the hippocampus, the higher the amplitude of GABAARs currents the better the cognitive performance score ($R^2=0.152$; $p=0.044$). A similar association was observed for AMPARs currents ($R^2=0.133$; $p=0.06$). The eE/I ratio was significantly higher in the TCx of AD subjects and was negatively associated with the MMSE in the TCx ($R^2= 0.205$; $p=0.059$) but not in the hippocampus. The synaptoproteome revealed the impact and directionality of protein alterations and neuropathology on the amplitude of synaptic receptors responses and cognitive MMSE scores.

Conclusions: These findings indicate that early shifts of the E/I balance contribute to the loss of cognitive capabilities in the continuum of AD symptomatology.
Aims: Glutamate is an amino acid and also the major synaptic signaling molecule of neurons in the brain, essential in learning, memory formation and cognition. Glutamate is neurotoxic. As soon as the glutamate signaling starts, it is stopped within 1 ms by astrocytes (which cover synapses), which take up and clear glutamate from the synapses, thus preventing extended signaling, which can impair synaptic function in glutamate neurotransmission, and lead to synapse loss and neuron cell death. Astrocytes express EAAT2 (excitatory amino acid transporter-2), the major glutamate transporter and 1% of brain protein. In Alzheimer’s disease (AD), astrocytes express less EAAT2. In experimental mouse AD models, increasing EAAT2 expression slows, and decreasing EAAT2 expression enhances disease progression and cognitive decline. These observations indicate EAAT2 as a novel drug target in AD.

Methods: Here we describe a simple assay to find drugs that can activate EAAT2 in glutamate uptake. The assay targets EAAT2 reconstituted in liposome membrane and measures glutamate uptake with red fluorescent Oxonol VI light emission.

Results: EAAT2 drugs may help in preventing and treating AD.

Conclusions: Drugs activating EAAT2 in glutamate uptake may help prevent and treat a variety of cognitive, neurologic and psychiatric disorders.
PROBING THE LATE-ONSET ALZHEIMER’S DISEASE RISK FACTOR PTK2B USING HUMAN (IPSC)-DERIVED NEURONS

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Aims: PTK2B (Protein Tyrosine Kinase 2β) is a Ca²⁺-activated non-receptor tyrosine kinase, identified by genome-wide association study (GWAS) as an important late-onset Alzheimer’s disease (AD) risk gene and has been described as a major player in the mouse hippocampal synaptic plasticity. In this work, we aimed at studying the possible effects of PTK2B in the neuronal electrical activity, taking advantage of human induced pluripotent stem cell (hiPSC)-derived neurons.

Methods: We are using hiPSCs CRRSPR-cas9-edited to produce PTK2B homozygous (KO) and heterozygous (HET) knockout clones. We evaluated the gene expression, cell type composition, and neuronal electrical activity using western blot, immunocytochemistry, and calcium imaging as a readout of the electrical function.

Results: Our preliminary data show that PTK2B under-expression in HET cells leads to an increase in the frequency of calcium spikes, without significant changes in the percentage of active neurons. In KO cells, this frequency is further enhanced, suggesting a dose-dependent effect of PTK2B in the regulation of neuronal activity. Interestingly, acute exposure to amyloid β increases the electrical activity in HET cells, but not in KO cells, which have already reached a high level of activity due to the deletion of the PTK2B itself.

Conclusions: Our preliminary data suggest that PTK2B regulates important biological processes involved in neuronal electrical activity and maturation under normal conditions, as well in an Alzheimer’s model. Thus, altered expression of this risk gene in the AD brain could play a role in the neuronal hyperexcitability observed in patients at the early stages of the disease.
BETA-ARRESTIN INVOLVEMENT IN DIFFERENTIAL EFFECTS OF ACUTE STRESS ON ABETA LEVELS IN MALE AND FEMALE MICE

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Aims: Objectives Almost 70% of people living with AD are female. Interestingly, stress-induced corticotrophin releasing factor receptors (CRF-Rs) signal differently in females and males. In females, CRF-Rs normally activate PKA/ERK. In males, CRF-Rs are withdrawn from the plasma membrane by beta-arrestin, resulting in significantly less CRF signaling. We hypothesize that the involvement of beta-arrestin in the stress signaling pathway in males underlies the differences in abeta levels in response to stress.

Methods: We used in vivo microdialysis to measure brain ISF Abeta levels every hour for several hours before, during, and after acute restraint stress in living APP transgenic mice. To study the influence of beta-arrestin1 on stress-induced changes in Abeta, we measured the effects of acute stress on Aβ levels in male and female beta-arrestin1 knock-out mice. To elucidate the CRF-signaling pathways, we used CRF, PKA, and ERK inhibitors before acute stress exposure.

Results: In females, acute restraint stress causes a rapid increase in brain interstitial fluid (ISF) Aβ levels in the hippocampus, whereas Aβ in males does not change. The increase in females is blocked by inhibiting the CRF receptor (CRF-R), PKA and ERK pathways. In male beta-arrestin1 knockout mice, stress increases ISF Aβ levels nearly identically to females.

Conclusions: Our data suggest that stress causes sex-dependent increases on Aβ and that are mediated by CRF-R/beta-arrestin signaling. Determining the cellular pathways that differ between the sexes could identify risks of developing AD and lead to therapeutics to specifically modulate the stress response in AD, potentially that vary by sex.
Aims: Mitochondria homeostasis alteration is a major early feature of AD (1). The AMP-activated protein kinase (AMPK) acts to promote mitochondrial health, and multiple AMPK targets are involved in various aspects of mitochondrial homeostasis, mitophagy, and inflammatory response. We investigated the impact of AMPK signaling cascade on mitochondrial dysfunctions, amyloid beta and Tau pathologies, neuroinflammation and cognitive impairments in AD.

Methods: We used in vitro AD study models accumulating Aβ and APP C-terminal fragments (APP-CTFs), ex vivo hippocampal organotypic slice cultures transduced with APPswe lentiviruses, and the 3xTgAD mice. We studied AMPK signaling cascade and analyzed mitochondrial functions and mitophagy using biochemical, imagery and flux cytometry approaches. We studied neuroinflammation in mice using immunohistochemistry and ELISA assays. We used pharmacological and genetic tools to modulate AMPK activity.

Results: We report alteration of the AMPK-ULK1 signaling cascade in in vitro, in vivo and human sporadic AD brains. Interestingly, repressing AMPK cascade amplifies APP-CTFs accumulation, impairs mitochondrial function, inhibits mitophagy and triggers dendrite shape alterations. Inversely, stimulation of the cascade shows beneficial effects by rescuing mitochondrial functions in vitro, increasing the number of mature dendritic spines ex vivo, and alleviating amyloid beta and Tau pathologies as well neuroinflammation and learning capacity impairments in 3xTgAD mice.

THE NA+/CA2+ EXCHANGER 3 IS FUNCTIONALLY COUPLED WITH THE NAV1.6 CHANNEL AND PROMOTES AN ENDOPLASMIC RETICULUM CA2+ REFILLING IN A TRANSGENIC MODEL OF ALZHEIMER’S DISEASE

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Aims: The remodelling of neuronal ionic homeostasis by altered channels and transporters is a critical feature of the Alzheimer’s disease (AD) pathogenesis. The activity of the Na+/Ca2+ exchanger (NCX), one of the main regulators of intracellular Na+ and Ca2+ concentrations ([Ca2+]i) was found to be functionally upregulated in AD brains, where it correlated with increased neuronal survival. In the present study we investigated any possible modulation of the NCX currents, the functional interaction between NCX and the NaV1.6 voltage-gated sodium channel, and their impact on Ca2+ homeostasis in a transgenic in vitro model of AD, the primary hippocampal neurons from the Tg2576 mouse, which overproduces Amyloid-Beta 1-42 oligomers.

Methods: we performed 1) electrophysiological recordings to assess NCX and Na+ inward currents, in the presence of siRNA and pharmacological inhibitors; 2) Ca2+ video-imaging with fluorescent probes to study intracellular [Ca2+]; 3) western blot and immunofluorescence analyses to assess NCX and NaV1.6 expression and subcellular localization, in primary hippocampal neurons from WT and Tg2576 mouse embryos.

Results: we observed the upregulation of a specific NCX isoform, NCX3, in the Ca2+ influx mode of operation, which contributed to enhance the endoplasmic reticulum Ca2+ content but not cytosolic [Ca2+]i in Tg2576 neurons compared to WT. Moreover, we found that the functional coupling between NCX3 and NaV1.6 channels, which had been found to be upregulated in Tg2576 neurons, was responsible for the increased NCX3 activity.

Conclusions: These findings indicate that NCX3 and NaV1.6 channels intervene in the Ca2+ remodelling occurring in Tg2576 hippocampal neurons.
INHIBITION OF HYPERFUNCTIONAL KV3.4 CHANNELS BY BDS-I RESTORES [CA2+]I TRANSIENTS AND ER CA2+ SIGNALING IN PRIMARY ASTROCYTES EXPOSED TO AMYLOID-BETA OLIGOMERS

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Aims: Intracellular calcium concentration ([Ca2+]i) transients in astrocytes represent a highly plastic signaling pathway underlying neuronal-astrocytic communication. In the last decades, growing evidence have suggested that the compromising of this phenomenon could contribute to the Alzheimer’s disease (AD) pathogenesis. Our previous findings revealed that the upregulation of the Kv3.4 voltage-gated potassium channel was involved in the astrocytic activation upon exposure to Amyloid-Beta 1-42 oligomers. Basing on this evidence, we investigated whether the functional modulation of Kv3.4 channels also affected astrocytic [Ca2+]i transients and endoplasmic reticulum (ER) Ca2+ homeostasis in primary astrocytes exposed to Amyloid-Beta 1-42 oligomers.

Methods: We performed 1) Western blot and immunofluorescence analyses to evaluate Kv3.4 and ER stress marker expression; 2) electrophysiological recordings to assess Kv3.4-mediated fast-inactivating K+ potassium currents, membrane potentials and spike frequency; 3) Ca2+ video-imaging to assess [Ca2+]i transients and ER Ca2+ content in the presence of a selective Kv3.4 inhibitor, namely the blood depressing substance-I (BDS-I) Anemonia sulcata toxin; 4) DCFH-DA fluorescence assay to measure ROS production.

Results: we found that the inhibition of the hyperfunctional Kv3.4 channels by its selective inhibitor BDS-I restored, in a dose-dependent way, [Ca2+]i transients, prevented the expression of caspase-12 and GRP78/BiP and counteracted the increased ROS production in astrocytes exposed to Amyloid-Beta 1-42 oligomers.

Conclusions: Our results showed that Kv3.4 channels are implicated in astrocytic dysfunction in response to Amyloid-Beta 1-42 injury while their inhibition appears to be protective. Hence, Kv3.4 channels as well as astrocytic excitability could represent a new target to explore in the search of new strategies for AD treatment.
Aims: Long-term activation of the unfolded protein response (UPR) leads to eIF2α-associated sustained repression of protein synthesis, Nrf2-related reduction of antioxidant response and cell death, as observed in the brain from Alzheimer disease (AD) patients. Down syndrome (DS) neuropathology shares many neuropathological features with AD including aberrant proteostasis and increased oxidative stress (OS). In this study, we aimed to identify the molecular mechanisms associating DS with the early chronic induction of the UPR induction, and with the development of proteotoxicity and redox dys-homeostasis in DS brain.

Methods: We analyzed the induction of the UPR in human brain from DS, DS-AD and AD subjects. Further by proteomics, we analyzed blood-derived cell from DS young to confirm the burden of DS genotype in promoting altered proteostasis. At final, we pharmacologically rescued PERK to discern its role in DS neuropathology.

Results: We found the early and sustained activation of PERK along with eIF2a-mediated inhibition of translation in young DS and AD subjects supporting their involvement in neurodegeneration. Surprisingly, we also found in DS the uncoupling between PERK and Nrf2 response, whose antioxidant effect is repressed early by a mechanism implicating the overexpression of Bach1. The pharmacological inhibition of PERK reduced eIF2α-driven repression of translation rescuing proteostasis, and promoted the rebalance of Nrf2/Bach1 ratio favoring redox homeostasis.

Conclusions: Our results suggest that the failure to regulate the PERK pathway may be an essential step to promoting cellular dysfunction in AD-like pathologies. These data also support the exploration of PERK inhibition as a therapeutic option for AD in DS.
POSTERS

UBA52 IS CRITICAL IN CHIP MEDIATED HSP90 UBQUITINATION AND NEURODEGENERATION: IMPLICATIONS IN PARKINSON’S DISEASE

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Aims: Objective: Parkinson’s disease (PD) is evidently related to α-synucleopathies suggesting the protein aggregation as one of the major pathological events, predominantly regulated by ubiquitin proteasome system (UPS). This study was focused to evaluate the role of genes encoding (UBA52, UBB, UBC, RSP27A) essentially required core component of UPS, ubiquitin in PD pathology.

Methods: Method: Both neuronal SHSY5Y cells and Sprage Dawley rats were exposed to neurotoxin rotenone to mimic the sporadic PD pathology. Cells were also transfected with wild-type human Myc-α-synuclein+/+ to mimic the diseased conditions. In silico predictions were made through utilizing online database. Experimentation includes the transfection of wild type human Myc-UBA52+/+ in cells to specify the role of UBA52 in disease pathogenesis along with other molecular assays.

Results: Employed models exhibited the depletion of UBA52 only, in conjunction with significant downregulation of tyrosine hydroxylase (TH) expression and neuronal death. In-silico data, mass spectrometric analysis and co-immunoprecipitation findings suggested the strong interaction of UBA52 with α-synuclein, HSP90 and E3 ubiquitin ligase-CHIP, besides its co-localization with α-synuclein in mitochondrion. In-vitro ubiquitylation assay indicated that HSP90 ubiquitylation essentially requires lysine-63 residue of UBA52 in presence of CHIP. Myc-UBA52+/+ overexpressed neuronal cells exhibited the downregulation of α-synuclein, increased TH and restored proteasome activity. Myc-UBA52+/+ overexpression further inhibited the disease related augmented HSP90 level and ER stress related neuronal death.

Conclusions: Conclusion: Taken together, our data highlights the crucial role of UBA52 in ubiquitination of HSP90 in parallel to its contribution in inflection of various disease related neurodegenerative signalling.
Aims: BIN1 is the second susceptibility gene for Alzheimer’s disease and presents more than 10 isoforms. This makes it difficult to assess its pathophysiological roles. To analyze this complexity, we developed a project: (i) to determine whether a differential neurotoxicity may occur as a function of BIN1 isoforms, (ii) to characterize the biochemical properties of the isoforms able to induce neurotoxicity and (iii) to dissect the mechanisms involved.

Methods: We took advantage of the Drosophila model to assess in vivo the impact of BIN1 isoforms on neuronal toxicity. We tested 3 representative isoforms: the brain isoform1 (BIN1iso1), the muscle isoform8 (BIN1iso8) and the ubiquitous isoform9 (BIN1iso9). We generated transgenic Drosophila expressing these isoforms and analyzed their toxicity in the Drosophila eye.

Results: We observed that BIN1iso1 expression specifically induced photoreceptor neuron degeneration with age. We showed that BIN1iso1 CLAP domain was necessary for BIN1iso1 toxicity. This domain is involved in endocytosis, a process related to the endosome-lysosome pathway. In addition, we showed that the degeneration was characterized by an accumulation of large vesicles harboring endosomal markers. We found that a loss of function of Rab5 (early endosome) and a gain of function of Rab11 (recycling endosome), abrogated photoreceptor degeneration.

Conclusions: Altogether, this indicated that an impairment of the endosome-lysosome pathway is a potential cause of the BIN1iso1-induced degeneration that we observed in our drosophila models. These results are of interest in AD since endosome enlargement is one of the first cytopathological marker observed in the disease.
Aims: Ubiquitin Proteasome system degrades the misfolded or damage protein, utilising various E3 ubiquitin ligases. RCHY1 is a RING-finger E3 ubiquitin ligase which ubiquitinates several proteins involved in DNA damage response and transcriptional factors. Considering evidences of amyloid beta aggregation and DNA damage in Alzheimer’s disease(AD) pathology. Here, in this study we explore the role of RCHY1 in AD related neurodegenerative signalling.

Methods: Both cellular (Neuro2A) and sporadic rat model (streptozotocin induced) of AD were employed for study. First the expression level of RCHY1 was correlated with AD related amyloid beta aggregation by immunofluorescence, thioflavin T and congo red staining. Co-immunoprecipitation and MS/MS studies were setup in both cellular and rat brain lysates to decipher the interacting proteins of RCHY1. Further the neuronal cells were silenced for RCHY1 through siRNA and correlated with amyloid aggregation, endoplasmic reticulum (ER) signalling and proteasome activity during diseased conditions.

Results: In both employed models, RCHY1 was significantly upregulated and well correlated with amyloid beta aggregation. MS-MS and co-immunoprecipitation data suggested the high interaction of RCHY1 with ER related chaperon GRP78. The diseased conditions exhibited the augmented level of ER stress markers like GRP78, GADD153, ATF-4 and caspase-12 along with neuronal death. Silencing of RCHY1 in neuronal cells, reverts the disease related alterations and inhibits the neuronal death. Moreover, siRNA-RCHY1 transfected cells showed the improved neuronal communication and restored proteasome activity.

Conclusions: Findings indicated that RCHY1 significantly contribute in AD pathology related amyloid aggregation, ER stress related signalling, neuronal communication and neuronal death.
THE REGULATORY EFFECT OF WUZI YANZONG PILL ON UPR SIGNALING PATHWAYS IN PARKINSON’S DISEASE MICE

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Aims: To explore the therapeutic effect of WYP on PD mice and its regulation mechanism on UPR signaling pathways.

Methods: PD mice were randomly divided into Normal, PD and WYP groups. WYP group were orally administrated with WYP for two weeks and the PD group were treated with same dosage of saline at the same time. Gait test were used to evaluate the behavioral performance. The positive expression of TH was detected by Immunohistochemical staining. The levels of GRP78, p-IRE1α, XBP1, p-PERK, p-eIF2α and ATF4 in brain were detected by Western Blot.

Results: The results showed that WYP treatment increased the left forelimb angle and findlimb stride length(P<0.05), decreased the total paw area and hindlimb stance width(P<0.05), accompanied by the improvement of TH positive cells(P<0.01). Further observation found that WYP treatment reduced the expressions of GRP78, XBP1, p-PERK, p-eIF2α(P<0.01), ATF4 and p-IRE1α(P<0.05).

Conclusions: Oral treatment with WYP can alleviate the behavioral and pathological changes in PD mice. Its mechanism may be related to the regulation of IRE1 and PERK signaling pathways. (Grants: NNSF of China 81102552 and 81703978, the Research Project Supported by Shanxi Scholarship Council(No.2021-142), the Young Scientist Cultivation Program Project, Shanxi University of Chinese Medicine (No.2021PY-QN-03), Central Government Guided Local Funding Projects for Science and Technology Development YDZX20201400001483, Returned Chinese Scholars Technology Activities Preferred Project, Shanxi Province of China 20200026, Natural Science Foundation of Shanxi Province 201901D111334, Key Research and Development Projects of Shanxi Province 201803D31209, Shanxi University Science and Technology Innovation Project 2019L0724.)
DIFFERENTIAL CONTRIBUTION OF THE AMYLOID PRECURSOR PROTEIN-DERIVED CATABOLITES TO MITOCHONDRIAL HOMEOSTASIS AND TRANSPORT IN ALZHEIMER’S DISEASE.

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Aims: Previous studies have identified mitochondria as a target of amyloid beta (Abeta) toxicity contributing to early cognitive decline and memory loss in Alzheimer’s disease (AD). Recent studies indicated that amyloid precursor protein-derived C-terminal fragments (APP-CTFs) are etiological triggers of AD [1], accumulating in mitochondrial-associated membranes [2]. We studied the effects of Abeta and APP-CTFs in mitochondrial structure, function, movement and mitophagy.

Methods: We used human neuroblastoma SH-SY5Y cells and primary neuronal cultures expressing the familial APPswe mutation or APP-CTFs, transgenic mice models accumulating Abeta and APP-CTFs (3xTgAD) or APP-CTFs only (2xTgAD) and adeno-associated-virus-C99 injected mice [3]. We modulated pharmacologically secretases activities, to discriminate between APP-CTFs and Abeta effects. We analysed a cohort of human post-mortem sporadic AD brains.

Results: APP-CTFs accumulation, independently of Abeta, induces mitochondrial fragmentation and cristae disorganization, mitochondrial membrane hyperpolarization and oxidative stress, coupled with mitophagic failure characterized by the activation and accumulation of autophagic markers and mitochondrial proteins and defective fusion of mitochondria with lysosomes. Importantly, APP-CTFs accumulation is correlated with a mitophagy failure phenotype in SAD human brains [3]. We recently unveiled the differential impact of APP-derived catabolites on mitochondrial transport machinery regulation.

PIRH2 REGULATES THE CYTOCHROME-C MEDIATED APOPTOSIS DURING ALZHEIMER’S DISEASE

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Aims: Pirh2 is a RING-finger E3 ubiquitin ligase which ubiquitinates several important cellular factors involved in various metabolic diseases, particularly cell cycle and DNA damage associated proteins. However, its functional role in Alzheimer’s disease (AD) is not well studied.

Methods: The study was setup to investigate the role of Pirh2 in disease related signalling employing both cellular Neuro2A and rat model of AD. The investigations were done to assess the role of Pirh2 in intrinsic apoptotic pathway focused on cytochrome-c translocation. Further the neuronal cells were transfected with siRNA-Pirh2 to assess its specific role in disease related signalling. Furthermore, the effect of Pirh2 on various biochemical alterations, DNA fragmentation and neuronal apoptosis was also evaluated during diseased condition.

Results: Both cellular and rat models of AD exhibited the upregulated Pirh2 which was well correlated with AD related pathological markers (acetylcholinesterase activity and p-Tau). MALDI-TOF/TOF and co-immunoprecipitation findings suggested the high interaction of Pirh2 with cytochrome-c along with other proteins related to mitochondrion signalling. siRNA mediated silencing of Pirh2 modulated the disease related pathological markers, alteration in mitochondrial functions (mitochondrial membrane potential & mitochondrial complex-I activity, calcium level and outer mitochondrial membrane located VDAC1) and ATP level suggesting its implications in energy biogenesis. Besides, the disease related DNA fragmentation and neuronal death were also inhibited in Pirh2 silenced neuronal cells.

Conclusions: Findings indicated the regulatory role of Pirh2 in cytochrome-c translocation, energy biogenesis and neuronal apoptosis suggesting its cardinal role in disease onset and progression.
CHARACTERIZATION OF MITOCHONDRIAL DYSFUNCTION IN BLOOD OF ALZHEIMER’S DISEASE PATIENTS

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**Aims:** Mitochondrial dysfunction is an early event in Alzheimer’s disease (AD) physiopathology and may contribute to neurodegeneration. The role of mitochondrial dysfunction in AD have been mostly explored in brain post-mortem tissues. These studies have particularly highlighted abnormal expression levels of mitochondrial fission and fusion protein. The balance between fission and fusion mechanisms is essential to maintain a pool of functional mitochondria inside the cell. Further studies suggest that this dysfunction may also be observed in immune cells, both in the central nervous system as in blood compartment. In this context, we investigate if mitochondrial dysfunction, in particularly, imbalance of mitochondrial fusion and fission could be detected in peripheral blood mononuclear cells (PBMCs) from AD patients compared to PBMCs from healthy control subjects.

**Methods:** Our study is a monocentric retrospective study, using blood derived PBMCs from AD patients and neurological controls follow at the Cognitive Neurology Center (CNC) in Paris. PBMCs were isolated and stored in Lariboisière hospital's biobank. Mitochondrial protein level were measured using immunoblot technique.

**Results:** No significant variation of fusion protein DRP1 was observed in PBMCs control vs AD. However, proteins involved in mitochondrial fusion such as MFN1 and MFN2 are significantly dysregulated in AD PBMCs compared to control subjects.

**Conclusions:** These new findings pave the way to explore the proteins implicated in mitochondrial homeostasis at peripheral levels, and may provide a new tool to follow the progression of mitochondrial dysfunction in AD.
Aims: Exploring the Influence of Adipokines on Neuronal Function in Alzheimer’s disease. Neha S Tomar, Sarah B Withers, Gemma Lace n.s.tomar@edu.salford.ac.uk, s.b.withers@salford.ac.uk, g.l.lace-costigan@salford.ac.uk Salford, M6 5TL 07405167200 School of Science Engineering and Environment, University of Salford, U.K. Mid-life obesity is a major risk factor for Alzheimer’s disease (AD). However, the molecular mechanisms responsible for obesity-driven pathophysiology are unknown. Adipocytes (fat cells) secrete signalling factors (adipokines) which have been shown to impact brain function; obesity is associated with oxidative stress and a dysregulated adipokine profile, which offers an insight into the mechanisms responsible. In this study we used retinoic acid-differentiated human neuroblastoma SHSY5Y cells and differentiated 3T3-L1 cells to investigate the effect of adipokines on neuronal-like cell viability. We also used patient-derived skin fibroblasts, as a surrogate of patient neuronal cells, to investigate the effect of individual adipokines.

Methods: Experiments were performed in the presence/absence of H$_2$O$_2$-induced oxidative stress. Viability was assessed using MTT and data are presented as mean ± SEM.

Results: The conditioned media and commercial adipokines showed protective effects on the neuronal-like cells with 95.1% ± 4.3 (*p<0.05) in comparison to the non-neuronal cells with 48% ± 8.09, and chemerin showed no protective effect in AD patient derived cells under H$_2$O$_2$ induced oxidative stress in comparison to the non-AD donors derived fibroblast cells (p>0.05).

Conclusions: Our findings suggested that the adipokines might play a vital role in neuronal-like cellular metabolic protection against oxidative stress. Further investigation of this could be fundamental in understanding the link between midlife obesity and Alzheimer’s disease.
Aims: The presence of cardiovascular (CV) risk factors may exacerbate neurovascular dysfunction associated to cerebral amyloid angiopathy (CAA) pathology. The mitochondrial are portrayed as sensors of cell damage and initiators of downstream pathways such as cell death and inflammation. In endothelial cells (ECs), mitochondrial dysfunction may lie as the point of convergence of CV risk factors and amyloid β (Aβ) induced neurovascular dysfunction. We have previously shown that carbonic anhydrase inhibitors (CAi) reduce amyloid-induced cell death and mitochondrial dysfunction. Here, we aim to understand the role of mitochondria in mixed vascular pathologies, such as in the presence of hyperhomocysteinemia (HHcy) and CAA. Furthermore, the potential therapeutic potential of CAi in vascular pathologies will be explored.

Methods: Human ECs were challenged with Aβ, Hcy, or the combination in the presence/absence of CAi. Mitochondrial dysfunction and blood-brain barrier (BBB) permeability were evaluated.

Results: Mitochondrial oxygen consumption rate (OCR) is affected in the presence of Aβ, and to a lesser extent by HHcy, and the CAi appear to prevent OCR dysfunction. The presence of Aβ or HHcy independently increased BBB permeability, and, when both present, HHcy worsened Aβ-induced barrier permeability; these effects were prevented by CAi.

Conclusions: BBB resistance is affected by the presence of Aβ, and HHcy aggravates Aβ-induced BBB permeability. The presence of CAi seems to attenuate both Aβ-induced BBB dysfunction and mitochondrial OCR. Our results demonstrate additive effects of amyloidosis and HHcy on BBB compromise in mixed vascular dementias, and a promising role for CAi as a therapeutic strategy against cerebral EC dysfunction.
FERROPTOSIS MECHANISMS IN DROSOPHILA MODELS OF ALZHEIMER’S DISEASE (AD)

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Aims: Ferroptosis is a recently discovered subset of regulated cell death characterised by glutathione (GSH) depletion, inactivation of glutathione peroxidase-4 (fly homologue PHGPx) and dysregulated redox homeostasis resulting in iron-dependent accumulation of reactive oxygen species (ROS). Although the ferroptotic mechanism remains largely unknown, it is closely associated with advancement of different neurological diseases including Alzheimer’s disease (AD), where both the dysregulated GSH system and inhibition of GPx4 are associated with pathogenesis. Mitochondria are the primary source of energy within neurons producing ROS as a byproduct and utilise GSH as their main line of defence against redox imbalance. Impaired mitochondria are vulnerable to oxidative damage and demonstrate higher favourability for ROS and not energy production. Ferroptosis may contribute to oxidative stress and impaired mitochondria seen in AD. The current study uses different molecular and behavioural assays to measure oxidative responses of fruit flies following manipulation of PHGPx, a central regulator of ferroptosis.

Methods: The morphology, size and number of mitochondria are compared in Drosophila following PHGPx over expression and knock down.

Results: We have found altering PHGPx expression may confer protection against AD pathogenesis including amyloid-beta toxicity, a key pathological hallmark of AD.

Conclusions: Although redox homeostasis and glutathione are disrupted in AD, whether ferroptotic mechanisms can be harnessed to offer brain protection from AD-associated toxicity has yet to be explored.
DELETERIOUS EFFECTS OF ALZHEIMER'S DISEASE-CAUSING PRESENILIN-1 MUTATIONS ON MITOCHONDRIAL DYNAMICS.

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Aims: Mitochondrial dysfunction and oxidative stress are observed in Alzheimer's disease (AD). It is already known that presenilin-1 (PS1) regulates its homeostasis and localizes to the mitochondrial membrane. Therefore, we examined how five PS1 mutations (A431E, E280A, H163R, M146V, and Δexon9) observed in familial AD (FAD) affect mitochondria.

Methods: We used H4 cell lines genetically engineered to express either the wild-type PS1 or one of the five PS1 mutants to examine mitochondrial homeostasis and functions. Furthermore, we used brains of PS1M146V knock-in mice, 3xTg-AD mice, and human AD patients to investigate the role of PS1 in regulating MAMs formation.

Results: Each PS1 mutant exhibited slightly different mitochondrial dysfunction. Δexon9 mutant induced mitochondrial fragmentation while A431E, E280A, H163R, and M146V mutants increased MAMs formation. A431E, E280A, M146V, and Δexon9 mutants also induced mitochondrial ROS production. A431E mutant impaired both complex I and peroxidase activity while M146V mutant only impaired peroxidase activity. All PS1 mutants compromised mitochondrial membrane potential and cellular ATP levels were reduced by A431E, M146V, and Δexon9 mutants. Through comparative profiling of hippocampal gene expression in PS1M146V knock-in mice, we found that PS1M146V upregulates Atlastin 2 (ATL2) expression level, which increases ER-mitochondria contacts. Moreover, ATL2 expression levels were significantly elevated in the brains of 3xTg-AD mice and AD patients.

Conclusions: Overall, our findings suggest that each of the five FAD-linked PS1 mutations has a deleterious effect on mitochondrial functions. The adverse effects of PS1 mutations on mitochondria may contribute to MAMs formation and oxidative stress resulting in an accelerated age of disease onset in people harboring mutant PS1.
MITOPHAGY AS A MOLECULAR CROSSTALK BETWEEN DOWN SYNDROME AND ALZHEIMER’S DISEASE

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Aims: Down syndrome (DS) adults present a high rate of Alzheimer's disease (AD) including early neuropathological hallmarks such as amyloid deposition and neurofibrillary tangle formation. Common intracellular mechanisms associated with these hallmarks include the trisomy of APP gene, present in the chromosome 21, and the dysfunction of autophagic and lysosomal pathways. However, limited information is available regarding other genes, apart from APP, related to premature AD development in DS. Our aim was to determine genes with the same expression profile shared in both conditions (DS and AD) that participate in autophagy and mitophagy.

Methods: Using a systems biology approach and meta-analysis of public databases we determine genes with the same expression profile in both conditions and validated their expression in induced pluripotent stem cells (iPSC) of DS and AD patients, in brain samples from transgenic AD mice (5xFAD), and postmortem brain samples from patients with AD.

Results: We found a list of genes overexpressed in both conditions, selecting the neighbor of BRCA1 gene (NBR1) that codifies for a selective autophagy receptor, as interest target. We validated NBR1 gene overexpression in DS and AD iPSC and in brain tissues of the AD transgenic mouse model, 5xFAD. Additionally, in postmortem brain tissues from AD patients, we observed an increase in the staining of the protein NBR1 together with the mitochondrial marker HSP70.

Conclusions: Conclusion: Our data pinpoint NBR1 as a new potential biomarker and/or therapeutic target for AD and propose that our systems biology approach can be experimentally replicated to improve future AD diagnosis.
Aims: Mitochondrial DNA (mtDNA) encodes proteins that are necessary for the production of cellular energy by the mitochondria. In neurons, shortage of this energy due to mitochondrial dysfunction triggers neurodegeneration. In our previous studies, we reported that subjects with pathogenic mutations that cause familial Alzheimer’s disease exhibit low content of cell-free mtDNA (cf-mtDNA) in cerebrospinal fluid (CSF) before the appearance of clinical signs, suggesting that a decrease in the CSF content of cf-mtDNA precedes the clinical signs of dementia. However, the source and the mechanisms of cf-mtDNA release are unclear. Moreover, the molecular mechanisms involved in regulation of mtDNA copy number and release by different genes that cause familiar Alzheimer’s disease are unknown.

Methods: To explore these questions, we have now investigated the effect of APP-Swe/Ind and PSEN1dE9 mutations on mtDNA replication, transcription and release in SH-SY5Y cell clones that permanently express these mutations using droplet digital PCR (ddPCR) and selfie ddPCR.

Results: We found that either APP-Swe/Ind or PSEN1d9 gene mutations both reduce mtDNA copy number, the amount of L-strand and H-strand mtDNA transcripts per cell, and the release of cf-mtDNA. In addition, we found that pharmacological inhibition of mitophagy enhances the release of cf-mtDNA in control cells, but not in cell clones expressing APP-Swe/Ind and PSEN1dE9 mutations.

Conclusions: These results indicate the presence of an mtDNA quality control system independent of mitophagy that is impaired by APP-Swe/Ind and PSEN1dE9 mutations. Moreover, the present results show that alteration of mtDNA dynamics is a common factor of different pathogenic mutations that cause Alzheimer’s disease.
MASS SPECTROMETRY IMAGING OF GANGLIOSIDES IN THE APP/PS1 MOUSE MODEL OF ALZHEIMER’S DISEASE

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Aims: Lipid dysregulation is a core component of neurodegeneration in Alzheimer’s Disease (AD). Gangliosides are members of the glycosphingolipid family enriched in the central nervous system. The healthy brain maintains a homeostatic balance of gangliosides. GM1, the most abundant ganglioside in the adult brain, exerts neuroprotective effects. In contrast, the accumulation of GM2 and GM3, degradative by-products of GM1, directly causes neurodegeneration. Therefore, ganglioside dysregulation is a key contributor in AD pathology. It’s unknown whether ganglioside dysregulation is worsened by, or contributes, to beta-amyloid (Aβ) accumulation in the brain. This project aims to investigate ganglioside dysregulation with aging in the amyloid precursor protein/presenilin 1 (APP/PS1) transgenic mouse model of AD that develops Aβ plaques. We hypothesize that Aβ accumulation leads to increased levels of simple gangliosides GM2 and GM3 contributing to neurodegeneration in AD.

Methods: Matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) was performed on wildtype and transgenic mice aged 4, 8, 12, and 18 months to quantify ganglioside distribution across brain regions.

Results: Our data demonstrate an age-dependent increase in simple gangliosides that are exacerbated in the Tg mice. Aged Tg mice showed higher levels of simple gangliosides GM2 and GM3 compared to Wt controls by 12 months of age. Significant differences between Tg and Wt were found in the cortex and hippocampus, brain regions of initial amyloid deposition.

Conclusions: Our work identifies the interplay between Aβ and simple gangliosides in driving neurodegeneration. The aging project design identifies the therapeutic window of opportunity to restore lipid balance and prevent neurodegeneration in preclinical AD.
NOVEL FUNCTIONS OF AD RISK GENE SORL1

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Aims: Rapidly accumulating evidence has implicated endosomal dysfunction as a central pathogenic event in Alzheimer’s disease (AD). Variants in the endosome-associated trafficking receptor SORL1 are highly associated with AD risk, suggesting aberrant endosomal trafficking of SORL1 cargo may underlie AD pathogenesis. Here, we sought to identify novel SORL1 cargo relevant to pathogenic processes in AD such as amyloid processing and tau phosphorylation to shed insight on how SORL1 dysfunction contributes to AD pathogenesis.

Methods: Mass spectrometry on SORL1 immunocomplexes from human brain tissue enabled the identification of novel SORL1 interactors. These interactions were confirmed in cell lines and human postmortem brain tissue through proximity ligation assay and co-immunoprecipitation. The functional consequence of SORL1 deletion was probed through deletion of SORL1 in cell lines using CRISPR/Cas9 followed by biochemical assays.

Results: We identify the reticulon protein RTN4 as a significant, novel binding partner of SORL1 in neurons and microglia. Further preliminary data suggests that SORL1 deletion influences endosomal trafficking of RTN4. Ongoing studies are investigating the role of the SORL1-RTN4 interaction in regulating amyloid and tau pathology.

Conclusions: These findings suggest a novel mechanism by which SORL1 dysfunction contributes to AD pathogenesis through mislocalization of RTN4. RTN4 activity and signaling through its receptor, NgR, have been implicated in both modulation of amyloid processing and tau phosphorylation. Future studies will explore this hypothesis further to understand how diminished SORL1-mediated endosomal trafficking can contribute to AD pathogenesis.
Aims: Brains of AD patients are characterized by the presence of amyloid pathology (A), tau pathology (T) and neurodegeneration (N). A role of ApoE in modulating these pathologies is increasingly emerging. This is strengthened by the identification of an EOAD-PSEN1-E280A mutation carrier with a homozygous ApoE3-Christchurch (R136S) mutation with delayed symptoms. This patient displayed high amyloid pathology, but low tau pathology and neurodegeneration. With ApoER136S considered as a loss of function mutation, we here aim to elucidate the specific contribution of ApoE to amyloid pathology and its downstream effect along the ATN axis.

Methods: We crossed 5xFAD mice with ApoE-/- mice and assessed amyloid pathology and microgliosis by immunohistochemistry. Now we are generating 5xFAD.TAU.APOE-/- mice, to assess the role of ApoE on amyloid facilitated tau pathology.

Results: We here demonstrate no changes in amyloid load at the age of 3 months in 5xFAD.ApoE-/- mice, compared to 5xFAD.ApoE+/+ mice. However amyloid pathology was qualitatively different in the absence of ApoE. Amyloid deposits appeared larger and more diffuse in ApoE KO mice. Plaque size was significantly larger and ThioS staining revealed significantly less fibrillar plaques in the absence of ApoE. Finally, microglia were less associated with amyloid pathology in the absence of ApoE. Crosses of 5xFAD.ApoE-/- mice with Tau transgenic mice are generated to assess the effect of ApoE on ATN pathologies.

Conclusions: Taken together, our findings indicate that ApoE has a modulatory role on amyloid deposition and morphology. We currently use this model to assess ApoE’s role on ATN pathologies.
Aims: Amyloid precursor protein (APP) processing occurs primarily in endosomal/lysosomal vesicles, the trafficking of which exerts a critical influence on the generation and deleterious effects of APP cleavage products, including β-amyloid peptide (Aβ). It is therefore not surprising that endolysosomal dysfunction is one of the first cellular features to emerge in the development of Alzheimer's disease (AD). Based on our previous work on neuronal membrane remodeling and associated protein dysfunction, we hypothesized that optimized dietary lipid intakes could help ameliorate functional alterations and in particular restore an efficient endolysosomal system.

Methods: Using the human neuroblastoma cell line SH-SY5Y overexpressing recombinant human APP carrying the Swedish mutation as a cellular model of AD, we investigated the endolysosomal system focusing on early endosome enlargement and impaired exosome secretion. Cells were exposed to different treatments capable of altering the lipid composition of the membrane.

Results: In Western blots combined with immunohistochemistry and nanoparticle tracking analysis, we observed that controlled amounts of docosahexaenoic acid (C22:6, n-3), a fatty acid known for its neuroprotective properties, restored endosome/lysosome trafficking as well as exosome secretion. These effects could be based on different specific interactions between APP cleavage fragments and certain endosomal proteins.

Conclusions: These results suggest the involvement of new mechanisms mediated by n-3 polyunsaturated fatty acids that may explain their beneficial role against neuronal cell death and the onset of AD.
Aims: Increasing evidence indicates that extracellular vesicles (EVs) play an important role in Alzheimer's disease (AD). Previously, we showed that young APP/PS1 mice have increased EV levels in their cerebrospinal fluid (CSF), which correlated with high amyloid beta (Aβ) levels at this age (Vandendriessche et al., 2021). Interference with EV biogenesis via pharmaceutical inhibition of neutral sphingomyelinase 2 (nSMase2) reduced EV release into the CSF and protected against acute Aβ oligomer-induced cognitive decline. Here, we aim to investigate nSMase2-mediated EV release in the APP\textsubscript{NLGF} knock-in mouse model.

Methods: APP\textsubscript{NLGF} mice were crossed with heterozygous nSMase2 knock-out (KO) mice (homozygous KO is not viable), of which the EV levels in the CSF were determined by ZetaView and ExoView. The APP\textsubscript{NLGF} x nSMase2\textsuperscript{+/−} mice were also analyzed for cognitive impairment (open field, novel object recognition, Y-maze) and Aβ plaque deposition (6E10 staining, ELISA).

Results: In contrast to APP/PS1 mice, ZetaView and ExoView analysis did not reveal increased EV levels in the CSF of young APP\textsubscript{NLGF} mice. Moreover, nSMase2\textsuperscript{+/−} did not translate in a reduced amount of EVs in the CSF in both the wild-type and APP\textsubscript{NLGF} condition. Although cognitive tests were inconclusive, we observed decreased plaque size in old APP\textsubscript{NLGF} x nSMase2\textsuperscript{+/−} mice, whereas the number of plaques stayed the same.

Conclusions: We plan to measure CSF-EV levels at different ages to gain insight into the CSF-EV profile along disease stages. We will perform the same analyses on APP\textsubscript{NLGF} mice crossed with nSMase2\textsuperscript{fl/fl} x Rosa26-CreERT2 mice after tamoxifen administration, which allows full body KO of nSMase2.
Aims: β-amyloid 1-42 peptides accumulation and aggregation is the principal hallmark for AD and it triggers to neuroinflammation onset activating Microglia M1 state stimulating pro-inflammatory cytokines which further enhance microglia to onset. For its properties and enzymatic activities, Transglutaminase 2 (TG2) could be taken in consideration as druggable marker of neuroinflammation AD-associated. Many evidences shows TG2 role in neuroinflammation AD-associated activating microglia and stimulating M1 markers production. To evaluate TG2 inhibition as possible therapeutic approach for AD neuroinflammation, we tested two Endocannabinoids, PEA and its oxazolinic derived, with documented anti-inflammatory power. We detected on TG2 and M1-M2 microglial markers expression to evaluate inflammatory state and define PEA and PEA-OXA neuroprotective power.

Methods: Mouse BV2 microglial cells were treated with fibrillated Aβ1-42 peptides in presence of PEA or PEA-OXA. Real Time PCR and Western Blot analyses were performed to evaluate TG2 expression and M1-M2 microglial markers.

Results: TG2 is up-regulated in presence of fibrillated Aβ 1-42 peptide, both protein and mRNA. In presence of PEA and PEA-OXA TG-2 protein and mRNA expression decreases considerably. M1 marker IL-6 expression has the same effect by endocannabinoids : its high Aβ 1-42 peptide-induced expression decrease in presence of PEA and PEA-OXA. Endocannabinoids are able to up-regulate M2 markers Arginase-1 and TREM2 expression.

Conclusions: Added to evidences in literature, these data confirm the possibility to consider TG2 as a marker of neuroinflammation. PEA and PEA-OXA has confirmed their anti-inflammatory role suggesting a possible use to block AD patients brain inflammation down-regulating TG2 expression and promoting microglial neuroprotective M2 state.
DIVERSITY OF PLAQUE-ASSOCIATED MYELOID CELLS SUBTYPES IN HUMAN ALZHEIMER’S DISEASE BRAIN

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Aims: Parenchymal microglia, as well other myeloid cells, have been postulated as a critical factor in Alzheimer’s disease (AD) pathogenesis since the identification of genetic risk factors related to their functions. However, the different phenotypes and the implication of the diverse immune cells in the human pathology have not been determined yet. In this work, we have further analyzed the phenotypic profile of the damage-associated myeloid cells in two AD vulnerable brain regions, the frontal cortex and hippocampus.

Methods: Immunohistochemistry and image analysis approaches have been carried out in postmortem brain samples from patients with AD (Braak V-VI) and aged controls without neurological symptoms (Braak II).

Results: Damage-associated microglial cells were clustered around amyloid plaques and expressed Iba1, TMEM119, CD68, Trem2 and CD45high. Moreover, AD brains exhibited parenchymal infiltration of CD163-positive monocyte-derived cells that invaded plaque near blood vessels. While the frontal cortex showed strong microglial activation similarly to that reported in amyloidogenic mice, the hippocampus of the same patients showed an attenuated microglial activation with a degenerative phenotype.

Conclusions: These findings suggest the existence of different myeloid populations associated with Aβ plaques that correlates with disease severity. These results open the opportunity to design targeted therapies, not only to microglia, but also to the population of macrophages to modulate amyloid pathology and provide a better understanding of the immunological mechanisms underlying AD progression. Supported by ISCiii of Spain grants PI18/01557 (AG), PI18/01556 (JV) co-financed by FEDER funds from EU , and by Junta de Andalucía grants UMA18-FEDERJA-211(AG), P18-RT-2233(AG) and US-1262734(JV) co-financed by Programa Operativo FEDER 2014-2020.
TOWARDS MANIPULATING MICROGLIAL SUBSETS IN HUMANS: APPROACHES TO POLARIZE MICROGLIA IN A TARGETED FASHION

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Aims: To build microglial in vitro models recapitulating identified in vivo microglial subtypes.

Methods: We have generated a single-cell RNA-sequencing dataset consisting of 212,000 microglial transcriptomes from purified, living human microglia from a broad array of neurological diseases including Alzheimer’s, ALS, Parkinson’s, MS, Epilepsy and individuals with no or mild cognitive impairment. We sampled multiple cortical and subcortical regions.

Results: Hierarchical clustering identified 12 microglial clusters with specific subsets associated with antigen presentation, motility, and proliferation. We further report an intriguing central divide of the identified subsets between oxidative and heterocyclic metabolism. Using the Connectivity Map (CMap) resource, we identified transcription factors, RNA-binding proteins and compounds associated with cluster-specific gene signatures. Following validation using internal single-nucleus RNA sequencing, published bulk RNA-sequencing and proteomic datasets from human microglia, we are using overexpression and CRISPR/Cas9 genome editing to genetically perturb different microglia models in vitro in order to validate the identified regulators. Comparison of cluster-specific gene signatures with the CMap resource also identified pharmacologic compounds associated with specific microglial subsets. The molecular signatures of our identified homeostatic-active clusters were associated with BRD-K39187410, an anti-amyloidogenic agent and the mTOR inhibitor Torin 2, while immunologically active clusters were associated with Camptothecin, a topoisomerase inhibitor. Preliminary gene expression data generated from a microglial cell line (HMC3 cells) treated with the identified compounds suggest the potential of these compounds to polarize microglia towards cluster-specific expression profiles.

Conclusions: Our data suggest that genetic and pharmacologic perturbation of microglia in vitro might enable the functional characterization of human microglial subtypes.
Aims: Microglial cells are resident macrophages of the central nervous system. Genome-wide association studies identified numerous Alzheimer’s disease (AD) risk genes highly or exclusively expressed in microglia, supporting the role of microglia as major players in AD pathogenesis. We aim to describe the effect of microglia activation on synaptic connectivity and its potential modulation by AD risk genes.

Methods: We developed a microfluidic co-culture device where fluidically isolated, mature neuronal synapses are brought in contact with microglia, enabling exclusive genetic and pharmacological interventions to neurons and microglia. We cultured cortical neurons from WT embryonic mice in pre- and postsynaptic chambers for 7 days, after which primary microglia from 10-month-old WT and APP (hAPPJ20, PDGFAPPSw,Ind) mice were added into the synapse chamber. Microglia morphology and synaptic connectivity near microglia were analyzed via immunocytochemistry and confocal microscopy.

Results: Exposure to lipopolysaccharide (LPS) activated microglia as evidenced by decreased surface area and increased circularity. Morphological changes will be correlated with Interleukin-1 concentration in the medium, indicative of an inflammatory response. We observed a higher sensitivity to LPS in microglia from APP mice compared to WT mice. However, the level of neuronal network disruption near microglia was similar for WT and APP mice.

Conclusions: In summary, our microfluidic co-culture device enables specific modulation and high-content analysis of microglial activity and synaptic connectivity in physiological and AD pathological conditions. On-going work is focused on live-cell recordings with fluorescent microglia to characterize their dynamic response to amyloid-β and on harvesting microglia from mice carrying mutations for microglia-related AD risk genes.
Aims: Gut microbiota, as a major environmental factor for Alzheimer’s disease (AD), has been observed to regulate microglial activation and amyloid pathology. However, the mechanisms of gut microbiota in modifying AD progression remain unclear. We hypothesized that GPR109a, a receptor for bacterial metabolites, might link the intestinal microbiota to AD pathology.

Methods: We cross-bred APP-knock-in (APP-KI) mice with GPR109a knockout mice.

Results: We observed that GPR109a deficiency substantially reduced cerebral amyloid β (Aβ) load, preserved synaptic structure proteins (e.g. Munc18-1, PSD95, synaptophysin and SNAP25) and improved cognitive function of AD mice. GPR109a deficiency affected neither the number of microglia nor the transcription of inflammatory genes, like tnf-α and il-1β; however, it increased microglia clustering around Aβ deposits in the brain of APP-KI mice, which might facilitate Aβ clearance. As a mechanism, GPR109a deficiency upregulated the transcription of trem2 gene, which might promote microglial response to the changes of extracellular environment. GPR109a deficiency also significantly increased the transcription of cx3cr1, p2ry12, and clec7a genes, which suggests the homeostatic status of microglia. Interestingly, GPR109a deficiency increased the expression of LRP1, a receptor transporting Aβ from the brain parenchyma to circulation and facilitating Aβ internalization by neurons and astrocytes.

Conclusions: Taken together, our results suggest that GPR109a deficiency attenuates amyloid pathology potentially by promoting microglia- and LRP1-mediated Aβ clearance, and reducing microglial neurotoxic inflammatory activation, both of which protect neurons in AD mouse brain.
SYNTHETIC AMYLOID BETA DOES NOT INDUCE A ROBUST TRANSCRIPTIONAL RESPONSE IN INNATE IMMUNE CELL CULTURE SYSTEMS

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Aims: The accumulation of amyloid beta (Aβ) in the brain has historically been associated with Alzheimer’s disease (AD), and recent evidence suggests that neuroinflammation also plays a central role in AD. These observations have led to the theory that Aβ induces proinflammatory activation of immune brain cells, which culminates in neuronal damage and cognitive decline. In order to test this hypothesis, many in vitro systems have been established to study Aβ-mediated activation of innate immune cells. Our aim is to study the transcriptional resemblance of these in vitro models to the microglia in the AD brain on a genome-wide scale.

Methods: We used bulk RNA-seq to assess the transcriptional differences between in vitro cell types used to model neuroinflammation in AD, including established, primary and iPSC-derived immune cell lines (macrophages, microglia and astrocytes) and their similarities to primary cells in the AD brain. We then analyzed the transcriptional response of these innate immune cells to synthetic Aβ.

Results: We found that human induced pluripotent stem cell (hIPSC)-derived microglia (IMGL) are the in vitro cell model that best resembles primary microglia. Surprisingly, synthetic Aβ does not trigger a robust transcriptional response in any of the cellular models analyzed, despite testing a wide variety of Aβ formulations, concentrations, and treatment conditions. Finally, we found that bacterial LPS/INFγ activate microglia and induce transcriptional changes similar to those observed in disease associated microglia present in AD.

Conclusions: These results suggest that the use of IMGLs treated with LPS/INFγ it’s a suitability in vitro model to study AD-related neuroinflammation.
PHENOTYPING IPSC MICROGLIA-LIKE CELLS WITH HIGH POLYGENIC RISK FOR ALZHEIMER’S DISEASE

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Aims: Late-onset Alzheimer’s disease (LOAD) has a substantial genetic component. Although the greatest risk factor for LOAD is the APOE gene, the disease is highly polygenic. Many genes recently identified by genome-wide association studies are highly expressed in microglia. To date, a small number of rare LOAD risk genes have been phenotyped in animal and induced pluripotent stem cell (iPSC) models, however the effect of common polygenic risk is unexplored. We aim to capture LOAD high polygenic risk in an iPSC resource generated from human patients, plus aged healthy controls with extremely low polygenic risk, and to comprehensively characterize microglia-like cells differentiated from these lines.

Methods: Frozen human PBMCs selected from the well-characterized Cardiff AD cohort will be reprogrammed to iPSC by Sendai vectors, and a single clone for each banked with EBiSC. Donors will be selected from LOAD patients with extremely high polygenic risk score (PRS: >+2), stratified by APOE alleles, and cognitively normal controls with extremely low PRS (<-2). iPSC will be differentiated to microglia-like cells in mass parallel, and run through a phenotypic screening pipeline to assess microglia health and function.

Results: We present current progress, and phenotypic screening assays that have been optimized in 96- and 384-well plate format to measure parameters including: morphology, endocytosis, phagocytosis, autophagy, mitochondrial health, inflammation, ATP-induced calcium flux, chemotaxis, lipid accumulation.

Conclusions: Generating iPSC from highly polygenic LOAD patients will allow us to assess phenotypic defects in isolated microglia-like cells, and determine whether there is commonality in the cell functions and intracellular pathways impacted.
CHARACTERIZATION OF A NOVEL IN VITRO BV2-MICROGLIAL CELL LINE OVEREXPRESSING HUMAN-APOLIPOPROTEIN E ISOFORMS.

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Aims: Apolipoprotein E4 (APOE4) is one of the major risk factors for the development of late-onset Alzheimer’s disease, and it is known to alter the microglial response during neuroinflammation. The molecular mechanisms by which the APOE genotype affects microglial responses during neuroinflammation are not yet fully determined. The BV2 murine microglia cell line constitutes a widely used cell line to study neuroinflammation. Additionally, Galectin-3 (Gal3) is a key molecule involved in microglial-mediated neuroinflammation and Alzheimer’s disease, and even though Gal3 and APOE share common ligands, such as Trem2, there are no pieces of evidence of an interaction between Gal3 and APOE in the CNS or in microglial cells. Thus, this project aimed to generate a novel BV2 mouse microglial cell line overexpressing the human APOE isoforms to better characterize the microglial molecular pathways affected by APOE during microglial activation by an inflammatory stimulus, including the evaluation of Gal3 inhibition, without the need to use primary cell cultures derived from APOE knock-in mice.

Methods: APOE-Plasmids were generated on a lentiviral backbone. An effective transduction was assessed by western blot and qPCR. BV2-APOE-transduced, and naïve BV2 cells were exposed to an inflammatory insult with lipopolysaccharide (LPS), amyloid-beta, and Gal3 inhibitors. The microglial response was evaluated by Griess reaction, qPCR, western-blot, Mitotracker and Lysotracker techniques.

Results: We expect to observe an effect of the APOE polymorphisms upon the inflammatory cascades triggered by LPS and amyloid-beta in BV2-APOE cells.

Conclusions: This novel vitro model will improve the understanding of the effects of the APOE polymorphisms in microglial function.
**POSTERS**

**LONG-TERM EFFECTS OF ACUTE SYSTEMIC INFLAMMATION IN ADULTHOOD ON MYELOID CELLS IN THE 5XFAD MOUSE OF ALZHEIMER’S DISEASE**

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**Aims:** Emerging evidence shows the close connection of immunity and susceptibility of Alzheimer’s disease (AD). Several studies have looked into the effects of systemic inflammation on microglia at very early or aged stage of brain. However, it remains to be clarified how this acute effect could lead to a long-term consequence in amyloid pathology. Therefore, we focus on alterations of microglia and monocytes after an acute systemic inflammation with LPS in 5xFAD mice before plaque deposition. We also want to investigate how systemic inflammation affects AD pathology.

**Methods:** One does of LPS (1mg/kg) was injected intraperitoneally in 6-week-old WT and 5xFAD mice. Cognition and memory were tested when they were 6-month-old. Mass spectrometry (MS) was used to analyze the proteomic signatures of microglia and monocytes from bone marrow. Immunohistochemistry was carried out to quantify amyloid load, activation of microglia, and neuronal activity.

**Results:** Quantitative MS revealed significant upregulation of MHC I was induced in microglia by either systemic inflammation or β-amyloid (Aβ). More proteins and bigger alterations were measured in monocytes than microglia. Gene Ontology analysis showed pyruvate metabolism and phagocytosis were mostly affected in monocytes due to Aβ pathology. No significant effect of LPS on cognition was observed in 5xFAD mice. Finally, we found reduced plaque burden and less activated microglia in LPS primed 5xFAD mice at region of dentate gyrus.

**Conclusions:** Our findings emphasize the potential long-term effects of early systemic inflammation which may contribute to mitigate Aβ load as a consequence.
Aims: The role of microglia in Alzheimer’s disease (AD) pathogenesis entails primarily the attempted removal of toxic materials, during which microglia transition into different phenotypes (ramified-homeostatic, hypertrophic-active and dystrophic-senescent), and hence, in its functions. Therefore, activating microglia and retaining them in hypertrophic state, as well as ablating dystrophic microglia has been postulated as promising therapeutic strategies for preventing neuroinflammation leading to further neurodegeneration. Here, we present a quantitative systems pharmacology (QSP) model that captures the pathology driven longitudinal changes in microglial phenotypic states and use it to explore pharmacological intervention to activate and preserve neuroprotective phenotype.

Methods: The model takes into account the above mentioned states of microglia. It was first calibrated with pre-clinical and clinical data to reproduce the longitudinal microglial dynamics, and then was applied to explore the pharmacological strategies to (i) activate and retain hypertrophic state and (ii) ablate dystrophic phenotype.

Results: The model suggests that subjects with certain microglial phenotype fractions would respond more efficiently to microglial activation therapies than others. For example, poor responders to microglial activation treatment are subjects who have greater than 50% dystrophic phenotype at the start of the treatment. For such cases, the model suggests that a combination with microglial ablation treatment targeting dystrophic cells or amyloid antibody treatment would be beneficial.

Conclusions: As far as we are aware, this is the first QSP model of microglial phenotype dynamics, which can be applied to guide drug development across various neurodegenerative diseases [5]. References [1] Chelliah et al., ASCPT 2022.
MODULATION OF SENILE PLAQUE PHENOTYPE BY ALPHA-SYNUCLEIN IN ALZHEIMER’S DISEASE KNOCK-IN MICE.

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Aims: Alzheimer’s disease (AD) is characterized by the presence of β-sheet enriched aggregates formed by amyloid-β (Aβ), α-synuclein (α-syn), and tau. Amyloid aggregates are neurotoxic and cause substantial synaptic degradation, damage, and loss of neurons, with subsequent memory loss. However, little is known about which aggregate structure contributes the most to neurotoxicity because experimental studies of AD depend on transgenic animal models that express human amyloid precursor protein (APP); however, these mice do not offer the possibility to study pathological crosstalk between amyloid proteins. The objective of this study was to research molecular mechanisms of amyloid crosstalk in vivo; for that, we crossed heterozygous APP knock-in mice with Swedish, Arctic, and Iberian mutations with A53T α-syn mice generating a bigenic mice that express two human proteins A53T α-syn and APPNL-G–F.

Methods: We performed histopathological and spectroscopic analyses to unravel the functional roles of α-syn in Aβ plaque formation.

Results: We successfully generated a novel bigenic model by breeding knock-in human APP with Swedish, Arctic, and Iberian mutations with the mouse that harbour human α-syn gene with A53T mutation. Our results demonstrate structural changes in the amyloid plaques accompanied by increased neuroinflammation in bigenic APPNL-G–F /α-syn A53Tmice.

Conclusions: APPNL-G–F /α-syn A53Tmice recapitulate key pathological features of AD-like pathology, such as a progressive accumulation of parenchymal amyloids and altered glial response. Specifically, we observed changes in amyloid plaque phenotype in APPNL-G–F /α-syn A53T mice. Thus, our findings support the potential use of this novel mouse model to investigate crosstalk of amyloid proteins relevant to AD pathogenesis.
BRAIN-WIDE CELLULAR RESOLUTION MEASUREMENT OF NEURONAL ACTIVITY, MICROGLIAL ACTIVATION AND AMYLOID DEPOSITION

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Aims: Recent advances in optical clearing and light sheet imaging have opened an exciting new avenue for brain-wide, cellular resolution immunostaining at the forefront of a dimensional shift from 2D to 3D histology. With access to the intricate anatomy of the whole intact brain, we are developing unbiased and complete pictures of brain pathology for pre-clinical studies.

Methods: With our optimized iDISCO-based clearing methods and our Mesoscale Imaging System for the ZEISS Lightsheet Z.1 microscope, we can image entire mouse brains in ~10 min. Further, our machine learning-enabled 3TK software identifies individual cells or protein aggregates throughout the brain and aligns them to the Allen Reference Atlas to produce a regionalized read-out of patterns across 100’s of brain areas.

Results: We have applied this technology to generate cellular resolution maps of neuronal activity by measuring the immediate-early gene (IEG) products Npas4 and cFos in response to both a light exposure task and in response to contextual fear conditioning. Further, we have optimized methods to identify and count microglia throughout the brain and perform regional analyses of microglial activation. Importantly, we have used Alzheimer’s Disease model 5xFAD mice to develop assays to generate brain-wide signatures of β-Amyloid deposition. Our automated analysis of microglia and β-Amyloid co-staining allows for brain-wide quantification of plaque-associated microglia.

Conclusions: These new methods for whole-brain, next generation 3D immunohistochemistry and anatomics are ideally suited to pre-clinical studies for unbiased, complete and anatomically precise mapping of the efficacy of CNS therapeutics. We thank NIMH for Grants R43MH119989 and R43MH122070.
LONGITUDINAL IN VIVO PET IMAGING OF P2X7 RECEPTOR IN THE APP/PS1-21 MOUSE MODEL OF ALZHEIMER’S DISEASE USING THE NOVEL RADIOTRACER [11C]SMW139

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Methods: APP/PS1-21 transgenic (TG) mice (N=7), and wild-type (WT) healthy control mice (N=7) had a baseline PET scan at 5 months old and three follow-up scans at 8, 11 and 14 months with [11C]SMW139. The same animals were imaged with [18F]F-DPA at 14 months. Expression of P2X7 and TSPO receptors was investigated using immunohistochemical staining.

Results: Time activity curves in TG and WT mice at baseline and follow-up scans indicated fast clearance and low retention of [11C]SMW139 in the brain neocortex during PET dynamic acquisition at 0-60 min. The averaged (3-15 min) standard uptake values (SUVs) showed similar [11C]SMW139 uptake in the neocortex and hippocampus of TG and their age-matched WT mice at baseline and all follow-up scans. On the other hand, averaged (25-50 min) SUVs showed higher [18F]F-DPA uptake in the neocortex and hippocampus of TG compared to their age-matched WT mice at 14 months. Findings from immunohistochemical staining did not show, in contrast to TSPO expression, notable change in the expression of P2X7 receptor with age.

Conclusions: Preliminary results of this study showed that [18F]F-DPA is better to monitor neuroinflammation in the APP/PS1-21 mouse model than [11C]SMW139. This may be explained by more limited expression of P2X7 compared to TSPO in this animal model.
MICROGLIAL INVOLVEMENT IN NEUROINFLAMMATION AND THE APPEARANCE OF NEUROFIBRILLARY TANGLES IN TAU PATHOLOGY

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Aims: This project focuses on unraveling the role of microglia in tau pathology and asserting how essential it is for neuroinflammation, and both appearance and spreading of tau tangles.

Methods: We used P301S mouse model for tau pathology and frontotemporal dementia to study microglia in the diseased brain. By means of immunohistochemistry, the aim is to characterize the neuroinflammation present in brain slices and how microglia-related inflammatory molecules are distributed. Moreover, Western Blot and Mesoscale ELISA plates are used to quantify the hallmarks of the disease in terms of amyloid beta, phosphorylated tau and neuroinflammation. On top of that, single-cell RNAseq analysis from microglial cells will be performed to further study their transcriptomics in this context. With BV-2, a murine microglia-like cell line, and primary cell cultures from mouse brains when exposed to full-length fibrillar tau protein we study how microglia from different backgrounds engulfs and spreads the pathological form of the protein and which pathways are activated by it.

Results: Expected results include an active role of microglia at spreading tau and expressing proinflammatory cytokines around the tangles. We also hypothesize that they shift towards a damage-associated phenotype in the disease. Moreover, a pattern of microglial RNA associated with pathology is expected in these mice, especially in terms of neuroinflammation and phagocytosis.

Conclusions: This project encapsulates the goal of better understanding how microglia is involved in dementia, expanding the knowledge about the connection between tau tangles and these cells.
POSTERS

A CO-CULTURE SYSTEM TO STUDY THE ROLE OF GENETIC VARIATION IN MICROGLIA AND ASTROCYTE CROSSTALK

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**Aims:** GWAS studies have implicated microglia genes in conferring risk for Alzheimer's disease (AD). Microglia activation can induce astrocyte transcriptomic changes. We have developed a human monocyte-derived microglia-like cells (MDMi) and mouse astrocyte co-culture system to address these questions. The system allows species specific gene expression assessment between microglia and astrocytes. Human samples used for MDMi are genotyped for clinically relevant single nucleotide polymorphisms while astrocytes can include mouse AD risk models.

**Methods:** Astrocytes are isolated from P1 mouse cortices using a GLAST positive separation. Human samples are genotyped for microglial AD risk SNPs. CD14+ monocytes are isolated and cultured with a cytokine cocktail inducing differentiation into MDMi in 10 days. qPCR assays are used to measure gene expression of microglia and astrocyte markers. Immunocytochemistry is performed to quantify cell types and culture purity.

**Results:** Poly-D-Lysine and Laminin coated and uncoated plates comparison indicated the co-culture grew best in uncoated wells. We verified that purified astrocytes could be pre-cleared with CD11B and O4 beads, cryopreserved, and subsequently cultured with sufficient viability with MDMi. We confirmed that human genes are not detected in mouse astrocytes and mouse genes are not detected in human MDMi.

**Conclusions:** This human rodent combination allows measurement of differential gene expression between human microglia and mouse astrocytes seamlessly in the same well through species specific primers and antibodies. We are now deploying this assay to look at human genetic variation's influence on astrocyte behavior with the addition of LPS or aggregated amyloid beta to stimulate activation and AD related cellular pathways.
HEXOKINASE 2 INHIBITION FINE-TUNE MICROGLIAL RESPONSE IN ALZHEIMER’S DISEASE

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Aims: In Alzheimer’s Disease (AD), inflammatory microglia meets its increased energy demand by reprogramming its metabolism. Hexokinase 2 (HK2), which catalyzes the first step of glycolysis, increases its expression specifically in microglia associated to Aβ plaques in AD. We hypothesize that microglial HK2 expression plays a key role in the neuroinflammatory response of microglia. Accordingly, we propose that the antagonism of HK2 could reduce disease progression by blocking its transition to a neurodegenerative phenotype.

Methods: We generated mice that have a conditional deletion of HK2 gene in microglial cells by crossing 5xFAD;CX3CR1-CreERT2 mice with mice harboring a floxed HK2 allele. Microglial deletion of HK2 was induced with tamoxifen at two months. Three months later, we assessed HK2 cortical levels, as well as several AD hallmarks. Similarly, we evaluated the acute effect of its pharmacological inhibition with Lonidamine. In order to ascertain the biological process affected by HK2 inhibition in AD, we performed transcriptomic analysis of the cortical samples with NanoString.

Results: The pharmacological inhibition of HK2 acts to modulate specific features of the microglial response, fine-tuning the resulting phenotype that displayed reduced microgliosis but sufficient barrier formation, increased uptake of Aβ and reduced expression of neurotoxic mediators. In vivo this translates in the preservation of synaptic proteins and improved cognitive performance. Similar results were obtained in HK2-haploinsufficient microglia in 5xFAD mice.

Conclusions: The striking effects observed after HK2 inhibition, strongly support the growing recognition that microglial metabolism is a critical determinant of its immune response during AD progression and a potential therapeutic target.
Aims: Dysregulated immune signaling in Alzheimer’s disease (AD) is characterized by abnormal microglial activity that can be detrimental to neuronal survival if sustained. Microglia in AD upregulate NFκB and NLRP3 inflammasome-mediated release of pro-inflammatory cytokines. To better understand the mechanisms driving this increase in microglia activity in AD, we investigated the role of extracellular vesicles (EVs) as propagators of microglia signaling. To blunt this signaling we tested potential anti-inflammatory effects of cannabidiol (CBD) on EV-mediated microglia activation.

Methods: EVs released from untreated or LPS treated cells were collected and used for RNA isolation to measure mRNA cargo. BV2-microglia cells were exposed to EVs released from untreated or LPS treated cells (500 ng/µl, 4000 EVs/cell) for 3 hours +/- ATP (1 µM) treatment. Cell lysates and RNA were collected after 3, 6, and 9 hours. In CBD experiments, cells were pre-treated with CBD (0.5 µM) for 3 hours.

Results: EVs derived from LPS-treated cells carry increased levels of IL1β and IL6 mRNA. Following treatment with LPS-treated EV, naïve cells significantly upregulate transcription of IL6, IL1β, iNOS and NLRP3. Western blot confirmed upregulation of pro-IL1β following LPS-EV treatment and active caspase-1 after additional ATP treatment. Pre-treatment with CBD significantly reduced transcription of IL1β and IL18 in EV treated cells.

Conclusions: EV-mediated pro-inflammatory signaling may represent a novel mechanism whereby AD-related enhanced microglial activity is propagated. Future work investigating EV-mediated propagation in vivo, and the potential role of CBD as a modulator of this activity, will improve our understanding of AD-related microglia signaling.
Aims: RNA-seq-based techniques used to describe the response of microglia to accumulating amyloid-beta plaques consistently report a disease-associated microglial (DAM) population, characterised by an upregulation of genes associated with inflammatory processes. Here, using the novel spatial transcriptomic technique, we explore the hypothesis that genes classically associated with DAM will be upregulated with specific spatial distribution in relation to plaques and whether this will be dependent on the Trem2 genotype.

Methods: FFPE hippocampal sections were prepared from 18-month AppNL-F (n=6) and AppNL-F/Trem2R47H (n=5) Knock-in mice alongside WT and Trem2R47H controls (n=4). After immunohistochemistry was performed, the sections were processed by the GeoMx digital spatial profiler (DSP; NanoString, Seattle WA). Whole transcriptome RNA-probes were collected from plaque-contacting, peri-plaque, and away-from-plaque regions. Bulk RNA-seq was performed using RNA collected from 18-month APPNL-F and WT controls (n=10-12).

Results: Many DAM-associated genes including those showing no significant change in bulk tissue, were increased only in plaque-contacting or peri-plaque regions. Notably, Trem2 expression in AppNL-F mice only increased in plaque contacting microglia as opposed to those in peri-plaque and away regions (P<0.01). As expected, this effect diminished with the introduction of the Trem2-R47H mutation. However, other genes such as ApoE showed a more graded change but again dependent on the Trem2 genotype.

Conclusions: Spatial transcriptomics exposes plaque-induced expression changes previously hidden in our bulk RNA-seq data. In particular, Trem2 effects occur at a distance from plaques, but its expression is only increased when the microglial cell is directly in contact with the plaque.
THE DARK MICROGLIAL SUBSET DISPLAYS ULTRASTRUCTURAL AND METABOLIC ALTERATIONS IN AN AGED MOUSE MODEL OF BETA-AMYLOID PATHOLOGY

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Aims: Recent technical advances helped unravel the considerable heterogeneity of microglia, the resident immune cells of the brain. One of these microglial subsets, the dark microglia (DM), is characterized by ultrastructural signs of cellular stress such as dilated endoplasmic reticulum (ER), altered mitochondria and loss of heterochromatin pattern. These dark cells were previously identified in middle-age wild-type (WT) mice as well as near amyloid beta plaques in age-matched APP-PS1 mice, an Alzheimer's disease (AD) mouse model. However, their ultrastructural features and interactions with AD hallmarks (plaques, dystrophic neurites) were not yet investigated in aging.

Methods: Using high-magnification chip mapping by scanning electron microscopy, we first analyzed the density and ultrastructure of both typical and dark microglia in the dorsal hippocampus CA1 (strata lacunosum-moleculare and radiatum) of 20-month-old male WT versus APP-PS1 mice.

Results: The density of DM profoundly increased in the APP-PS1 mice compared to WT controls, comprising nearly 20% of all microglial cells present in the stratum lacunosum-moleculare. We found DM interacting more with dystrophic neurites, while contacting less healthy synaptic elements and myelinated axons compared to typical microglia. Moreover, DM possessed more dilated ER and Golgi apparatus, as well as more mitochondrial alterations, alongside a cytoplasmic accumulation of glycogen granules.

Conclusions: The present study further highlights the close interactions between DM and AD hallmarks, suggesting a specialized involvement, whether beneficial or detrimental, compared to typical microglia in this context. Further investigations into the prevalence and parenchymal interactions of DM with AD hallmarks will be performed in post-mortem brain samples from AD patients.
CELL ADHESION MOLECULES REGULATE MICROGLIAL CHEMOTAXIS AND ALLEVIATE ALZHEIMER’S DISEASE PATHOLOGY

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Aims: Impaired microglial clearance reduces turnover of amyloid-beta (abeta) and leads to the accumulation of abeta plaques in Alzheimer’s disease (AD). While microglial clearance of abeta can be enhanced by interleukin (IL)-33 through inducing a phagocytic microglial subpopulation, its underlying mechanisms remain largely unclear. Thus, we aim to examine the molecular pathways that initiate the microglial clearance of abeta.

Methods: We performed single-cell and bulk transcriptomic profiling of microglia upon IL-33 treatment in APP/PS1 mice. Also, we generated genetic ablation of IL-33 receptor ST2 and cell adhesion molecule (CAM) in APP/PS1 transgenic mice.

Results: Our findings demonstrate that IL-33 enhances microglial clearance activity through stimulating a stepwise state transition in AD. Along the developmental lineage of IL-33-responsive microglia, microglia first transit from homeostatic state to chemotactic state and finally adopt phagocytic state. Inhibiting the IL-33-induced chemotactic state in microglia abolishes the subsequent development of phagocytic state and abeta clearance. Furthermore, we showed that induction of a CAM in chemotactic microglia regulate their chemotaxis towards abeta plaques upon IL-33 treatment. Inhibiting the CAM activity, by genetic ablation and neutralizing antibody, abolishes the microglial abeta chemotaxis and clearance stimulated by IL-33.

Conclusions: We demonstrate that induction of CAM in microglia enhances microglial chemotaxis and clearance of abeta, which leads to beneficial outcomes in AD.
LPS DISTINCTIVELY CAUSES A TRANSCRIPTIONAL SHIFT IN IPSC-MICROGLIA SIMILAR TO AN ACTIVATED STATE FROM GENETIC MOUSE MODELS OF AD

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Aims: Alzheimer's disease (AD) is the most common form of dementia and risk-influencing genetics implicates microglia and neuroimmunity in the pathogenesis of AD. iPSC-microglia are increasingly used as a model of AD but the relevance of common immune stimuli to model AD is unclear. We performed a detailed cross-comparison over time on the effects of combinatory stimulation of iPSC-microglia, and assessed their relevance to AD using both human post-mortem and mouse model data.

Methods: Using single cell RNA-seq we measured the transcriptional response of human iPSC-microglia after 24 and 48h of stimulation with PGE₂ or LPS+IFN-γ either alone or in combination with ATPγS. For comparison, we performed a transcriptomic meta-analysis of microglia from AD mouse models.

Results: We observed a shared core transcriptional response of iPSC-microglia to ATPγS and to LPS+IFN-γ, suggestive of a convergent mechanism of action. Across all conditions tested in human iPSC-microglia we observed a significant overlap and functional links to genes that change their expression levels in microglia from AD patients. Using a data-led approach, we identify a common axis of transcriptomic change in mouse microglia across a range of AD genetic mouse models also able to segregate homeostatic from activated response microglia described in AD mouse models characterized with Aβ accumulation. Finally, we show that only LPS provokes a transcriptional response along this axis in mouse microglia and LPS+IFN-γ in human iPSC-microglia.

Conclusions: A data-led comparison of the human iPSC-microglia transcriptional responses to the AD genetic mouse models proposes LPS as the most relevant immune provocant for AD models.
WHITE MATTER (WM) ABNORMALITIES CORRELATE WITH CSF BIOMARKERS OF MICROGLIAL ACTIVATION AND PATHOLOGICAL TAU IN ALZHEIMER’S DISEASE

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Aims: Activated microglia as a function of beta-amyloid (Aβ) induced neuroinflammation have been implicated in the spread of hyperphosphorylated tau in Alzheimer’s disease (AD). Microstructural alterations in white matter (WM) bundles connecting regions with Aβ and tau aggregates have also been detected in AD. The purpose of this study was to investigate the relationship between CSF biomarkers of activated microglia and pathological tau, and WM markers of neuroinflammation and myelin content in AD.

Methods: Post-hoc analyses of baseline proteomics and free-water corrected diffusion tensor MRIs from a phase 1b, open-label study of XPro1595 in patients with AD (n=16) and peripheral markers of inflammation (ADi) were conducted. CSF samples were analyzed using the Olink® Target 48 Cytokine panel, Roche Elecsys® NeuroToolKit (NTK) and Proteome Sciences TMT® Calibrator™. Proteomics data were screened for detection of CSF biomarkers known to increase in AD and to strongly correlate with standard uptake value ratios (SUVRs) of a PET ligand ([¹¹C]PBR28) recently validated as an indicator of activated microglia in AD.

Results: CSF biomarkers of activated microglia and pathological tau detected in quantifiable levels included: TNFS10, TGF-alpha, sTREM2, CSF1, VEGFA, IL10, CCL3, and p-tau-217, among others. CSF concentrations of these biomarkers correlated with MRI indicators of neuroinflammation (free-water) and myelin damage (radial diffusivity) in WM bundles known the be affected in AD.

Conclusions: CSF biomarkers of activated microglia and pathological tau are associated with WM abnormalities in ADi. Further research into WM abnormalities and the spread of pathological tau in ADi is thus warranted.
SOLUBLE ABETA INCREASES CYTOSOLIC AND MITOCHONDRIAL CALCIUM IN ASTROCYTES IN VIVO

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Aims: Amyloid β (Aβ) plaques are one of the main hallmarks of Alzheimer's disease (AD). Using in vivo multiphoton microscopy, we have previously reported that cytosolic resting calcium (Ca²⁺) is globally elevated in astrocytes in a transgenic mouse model of cerebral β-amyloidosis (APPswe/PS1dE9), a phenomenon that is independent of the astrocyte proximity to Aβ plaques. Thus, we aimed at investigating how Aβ oligomers (Aβo) (rather than Aβ plaques) contribute to the Ca²⁺ insult in astrocyte cytosol and mitochondria in vivo.

Methods: We topically applied naturally secreted soluble Aβo (conditioned medium from cultured transgenic primary neurons) onto the brain surface of 4-6-mo-old wild-type mice. Using a ratiometric genetically-encoded FRET-based Ca²⁺ indicator (Yellow Cameleon 3.6) targeted to astroglial cytosol or mitochondria, we investigated changes on astrocyte and mitochondrial Ca²⁺ levels respectively relative to baseline via intravital multiphoton imaging in vivo.

Results: Ca²⁺ levels dramatically increased in astrocytes upon topical soluble Aβo application, in all cytosolic astrocyte compartments (soma, branches and end-feet), but also in mitochondria, which in excess (i.e. mitochondrial Ca²⁺ overload) could be harmful to the cells. Aβ-immunodepleted transgenic conditioned media and wild-type conditioned media did not alter astrocyte cytosolic or mitochondrial Ca²⁺, supporting the specificity of the observed effects.

Conclusions: These results support a detrimental role of Aβo which leads to astrocyte Ca²⁺ dyshomeostasis in vivo, implying that Aβo are also involved in the astrocytic dysfunction observed in AD. Future studies will address the source of soluble Aβo-induced Ca²⁺ increase, including intracellular Ca²⁺ stores and channels involved, which could have therapeutic value.
CROSSTALK BETWEEN ASTROCYTES AND MICROGLIA RESULTS IN INCREASED DEGRADATION OF ALPHA-SYNUCLEIN AGGREGATES

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Aims: Compelling data indicate that neuroinflammatory cells, including astrocytes and microglia, play a central role in the pathogenesis of Parkinson's disease (PD). However, how the interplay between the two cell types affects their clearing capacity and consequently the disease propagation, remains unclear. The aim of the present study was to investigate in which way glial crosstalk influences alpha-synuclein (αSYN) pathology, focusing on accumulation and degradation.

Methods: For this purpose, hiPSC-derived astrocytes and microglia were exposed to sonicated αSYN fibrils and analyzed over time. The capacity of the two cell types to clear extracellular and intracellular protein aggregates when either cultured separately or in co-culture was studied using immunocytochemistry and ELISA. Moreover, the capacity of cells to interact with and process protein aggregates was tracked using time-lapse microscopy.

Results: Our data show that intracellular deposits of αSYN are significantly reduced in co-cultures of astrocytes and microglia, compared to monocultures of either cell type. Moreover, co-cultured astrocytes and microglia are in constant contact with each other via tunneling nanotubes and other membrane structures. Notably, our live cell imaging demonstrated that microglia, when attached to the cell membrane of an astrocyte, can attract and clear intracellular protein deposits from the astrocyte.

Conclusions: Taken together, our data demonstrate the importance of astrocyte and microglia interactions in αSYN clearance, highlighting the relevance of glial cellular crosstalk in the progression of PD-related brain pathology.
THE ROLE OF APOLIPOPROTEIN E (APOE) IN HUMAN ASTROCYTES PHYSIOLOGY

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Aims: The major genetic risk factor for Alzheimer’s disease (AD) is the presence of apolipoprotein E4 (APOE4) allele. In the brain, APOE is mainly expressed by astrocytes and plays an important role in the transport of cholesterol and other lipids, as well as neuronal growth, energy metabolism, synaptic plasticity, and immune response. While APOE4 confers a higher risk for developing AD, APOE2 is known to lower the risk while APOE3 is defined as average risk for AD. Despite ongoing research, the exact mechanisms of how the different APOE isoforms regulate astrocyte homeostasis in the brain is still poorly understood.

Methods: In this study, we generated human induced pluripotent stem cell (iPSC)-derived astrocytes (iAstrocytes) with hemizygous expression of APOE2, APOE3, APOE4 or APOE-KO to study APOE isoform-dependent changes in astrocyte physiology. We have further performed a label-free mass spectrometry-based proteomic analysis of APOE2, APOE3, APOE4 and APOE-KO iAstrocytes.

Results: Proteomic GO enrichment analysis showed APOE genotype-dependent changes in pathways including energy metabolism, more specific in lipid and glucose metabolism and aerobic respiration. APOE4 cells showed higher degree of aerobic respiration compared to APOE2 and APOE3 cells, whereas a higher degree of lipid biosynthetic processes was observed in APOE4 cells compared to APOE2 and APOE3 cells. Glucose homeostasis was found to be upregulated in APOE2 cells compared to APOE3 and APOE4 cells.

Conclusions: These new proteomic data of APOE isogenic iAstrocytes indicate an APOE genotype-dependent regulation of energy metabolism which needs to be further investigated by applying functional assays in future.
VENULAR AMYLOID DEPOSITION Follows Cerebral Amyloid Angiopathy in the F344-TGAD Rat

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Aims: Amyloid deposition in cerebral vessel walls is comorbid with AD. Current CAA research primarily focuses vascular Aβ deposition to be associated with arterial walls, while venular amyloid deposition has yet to be fully characterized. We sought to understand venular amyloid by identifying the Aβ peptide and studying the interplay between venular and arterial amyloid association.

Methods: 40 micron coronal brain sections were taken from TgF-344 AD rats aged 6-15 months of age. Immunofluorescent staining for Aβ was performed using a battery of antibodies spanning the entire Aβ sequence, in conjunction with markers specific for arteries, veins and capillaries. Ab deposits were quantified using image analyses across all ages. Microdissection microscopy isolated arterial and venular samples for mass spectrometry analyses to identify proteins within thioflavin S positive deposits.

Results: Immunofluorescent staining using multiple Aβ-specific antibodies further confirmed localization of Aβ along penetrating cerebral veins and offered a comprehensive visualization of vascular Aβ morphology. Proteomic analysis using mass spectrometry (MS) of amyloid deposits isolated from TgF344-AD rat brains by the laser capture microdissection (LCM) identified Aβ in venular amyloid but in relatively lower abundance than arterial amyloid and parenchymal plaques. Furthermore, various classes of proteins (i.e., tubulin, GFAP, 14-3-3, ApoE) were also identified in the MS analysis of cortical parenchymal plaques, arterial and venular amyloid.

Conclusions: Gaining a comprehensive understanding of the mechanisms involved in venular Aβ deposition and associated pathologies will provide insight into the connection between CAA and AD.
Aims: Alzheimer’s disease (AD) is a devastating neurodegenerative disorder resulting in the progressive decline of an individual’s cognitive abilities. Synaptic loss has provided the best neurophysiological correlate of cognitive decline to date however the importance of glial cells, specifically astrocytes, has gained recent attention in the literature. Changes in astrocyte function have been observed in AD patients and animal models contributing to neuronal death and synapse loss. In fact, astrocyte-derived neurotoxins mediate the neurodegeneration in AD. Neuron-focused research in AD has been unsuccessful in finding a treatment, therefore targeting reactive astrocytes and their involvement in brain inflammation may represent a potential therapeutic pathway.

Methods: Using an AAV vector, the proneural transcription factors, ASCL-1 or mutant ASCL-1, were expressed under a GFAP promoter in 14-month-old TgF344 AD rats. AAV2/5 vectors were unilaterally injected into the hippocampal hilus. Over the course of 7 weeks, rats were evaluated for electrophysiological and behavioral data, and eventually sacrificed to examine pathology and histology.

Results: TgF344 AD rats have GABAergic and glutamatergic neuronal loss starting at 13 months of age. Immunohistochemical analysis revealed that the expression of proneural transcription factors resulted in a 4-fold increase in neurons, NeuN+ cells, with a 40% loss in reactive astrocytes, GFAP+ cells. The correlation between these changes in pathology/cellular composition with functional and cognitive measurements, including electrophysiological recordings, spatial memory, and executive function, will be presented.

Conclusions: Astrocyte reprogramming leads to the generation of functional neurons, improved AD pathology, enhanced neuronal survival, and decreased gliosis.
ASSOCIATION OF Aβ PLAQUES AND ASTROGLIAL CONNEXINS IN THE SPINAL CORD OF THE 5XFAD MOUSE MODEL OF ALZHEIMER DISEASE

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Aims: Alzheimer’s disease (AD) is a progressive neurodegenerative disorder with memory loss, cognitive and mobility decline. Central nervous system (CNS) glia, particularly astrocytes and oligodendrocytes are linked to the pathogenesis of Alzheimer’s. Glial cells are coupled through gap junctions (GJ) assembled by two hemichannels consisting of six connexin (Cx) proteins on each opposed cell. Recent studies support that connexins might play a pathological role in Alzheimer’s progression. The aim of this project is to investigate aspects of Alzheimer’s pathology in the spinal cord of the 5xFAD mouse model.

Methods: Motor performance tests were performed in 5XFAD and non-transgenic mice groups (3 and 12 months). Fluorescence immunohistochemistry (IHC) in 3rd-4th cervical and 2nd-3rd lumbar frozen spinal sections was carried out for 6E10, Cx43, Cx30, GFAP, and Iba1 markers.

Results: Twelve-month-old 5XFAD mice showed a significant impairment in all motor performance tests. Also, they showed abundant extracellular Aβ plaque deposition in spinal cord which exhibited a time dependent increase. Older 5xFAD mice showed increased immunoreactivity for GFAP+ astrocytes and Iba1+ microglia compared to 3-month-old mice. Lastly, 12M 5XFAD mice exhibited increased immunoreactivity of Cx43 and Cx30 around and below Aβ plaques in the grey matter of the abovementioned spinal levels.

Conclusions: We detected severe astrogliosis which may contribute to Alzheimer’s pathogenesis. Future studies will be conducted for the investigation of connexins’ contribution to the spinal synaptic loss, myelin and to the motor deficiencies in 5xFAD mice.
INVOLVEMENT OF PURINERGIC RECEPTORS IN ASTROCYTIC CA2+ DYNAMICS IN ALZHEIMER’S DISEASE CONDITIONS

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Aims: Considering that astrocytes involvement in Alzheimer’s disease (AD) remains to be clarified, the present work aims to investigate how AB peptides affect astrocytic Ca²⁺ dynamics and their modulation by P₂X₇ (P₂X₇R), P₂Y₁ (P₂Y₁R) and adenosine A₂A (A₂AR) receptors.

Methods: We performed live-cell Ca²⁺ imaging and intracellular [Ca²⁺] ([(Ca²⁺)]) measurements, using a fluorescent probe, in cultured astrocytes stimulated with ATP (100 µM), BzATP (P₂X₇R agonist, 100 µM) or MRS2365 (P₂Y₁R agonist, 30 µM). Astrocytes were incubated with AB₁-₄₂ (1 µM, for 1 h) to mimic AD conditions, and further exposed to antagonists SCH58261 (A₂AR, 50 nM), JNJ47965567 (P₂X₇R, 1 µM) and MRS2179 (P₂Y₁R, 30 µM) and PKA inhibitor H-89 (10 µM).

Results: AB₁-₄₂ exposure significantly reduced astrocytic Ca²⁺-response amplitude to ATP stimulation, but increased its duration compared with control astrocytes. Cells pre-incubated with AB₁-₄₂ and exposed to P₂Y₁R antagonist decreased Ca²⁺-response amplitude (p<0.05) but not duration, whereas A₂AR antagonist, SCH58261, prevented AB-induced duration decrease (p<0.01). Curiously, SCH58261 decreased [Ca²⁺] evoked by BzATP and MRS2365 in control cells (p<0.05), being devoid of effects in Aβ-exposed astrocytes. Stimulation with P₂Y₁R agonist significantly decreased (p<0.01) Ca²⁺-response in the presence of P₂X₇R antagonist, which was not observed in Aβ-treated cells. Moreover, PKA inhibitor reduced BzATP- and MRS2365-mediated Ca²⁺-response in control astrocytes.

Conclusions: AB₁-₄₂ affected amplitude and duration of ATP-evoked Ca²⁺-response through a mechanism involving P₂Y₁R and A₂AR, respectively. Moreover, our data suggest a A₂AR/P₂Y₁R crosstalk in control astrocytes that was absent in AB-exposed cells, which might underlie astrocytic Ca²⁺ deregulation under AD-like conditions.
ATP RELEASE BY ASTROCYTES EXPOSED TO AMYLOID-BETA IS MODULATED BY A CROSSTALK BETWEEN ADENOSINE A2A RECEPTORS AND CONNEXIN 43

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Aims: Increasing evidence implicates astrocytes in Alzheimer’s disease (AD), a neurodegenerative disorder characterized by memory loss that parallels extracellular amyloid-beta (Aβ) peptides accumulation. Aβ affects astrocytes function, namely their capacity to release neuroactive molecules. Astrocytes release ATP, which can be metabolized into adenosine by ecto-5'-nucleotidase, CD73, resulting in adenosine A2A receptors (A2AR) activation that in turn bolsters neurodegeneration. AD's brains exhibit increased levels of A2AR and connexins 43 (Cx43), which form astrocytic hemichannels (Cx43-HC) that mediate gliosignals release. Objective: Investigate a possible association between Cx43-HC and A2AR in Aβ-challenged astrocytes.

Methods: Primary cultures of astrocytes were exposed to Aβ₁₋₄₂ peptides (1μM). ATP extracellular levels were evaluated resorting to a bioluminescent assay and hemichannels activity through ethidium bromide uptake. Cx43 levels and association with A2AR were assessed by Western-blot, co-immunoprecipitation and proximity ligation assays.

Results: We observed an increase in ATP extracellular levels triggered by Aβ₁₋₄₂ (161.7±12.2% vs. control:100%, p<0.001), mainly through Cx43-HC, prevented by A2AR antagonist (SCH58261, 50nM, 86.5±14.3%, p=0.0001). HC activity was regulated by A2AR, as A2AR antagonism counteracted the Aβ₁₋₄₂-triggered augment in HC permeability (Aβ₁₋₄₂:129.0±3.9% vs. SCH58261+Aβ₁₋₄₂:103.7±6.4%, p<0.001). Likewise, CD73 inhibition (AOPCP, 100μM) prevented the increased HC activity and ATP release caused by Aβ₁₋₄₂. Furthermore, A2AR blockade prevented Cx43 upregulation and phosphorylation in Aβ₁₋₄₂-exposed astrocytes, being observed that A2AR were closely associated with Cx43.

Conclusions: Our data identified a feed-forward loop involving A2AR and Cx43-HC in Aβ-challenged astrocytes, whereby A2AR overfunction increases Cx43-HC activity and ATP release that is converted into adenosine, by CD73, and sustains the increased A2AR activity.
HUMAN PLURIPOTENT STEM CELL-DERIVED ASTROCYTES FROM ALZHEIMER’S DISEASE PATIENTS FOR DISEASE MODELING

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Aims: The lack of reliable models of Alzheimer’s disease (AD) has impeded the development of effective therapies. Glial cells have a key role in AD pathology, but this cannot be properly modeled using available animal models, so we hypothesized that cells derived from Alzheimer’s patients can serve as a better platform for studying the disease. In this sense, human pluripotent stem cells (hPSC) allow the generation of different types of neural cells, which can be used for disease modeling, identification of new targets and drugs development.

Methods: We have a collection of hiPSCs derived from patients with sporadic forms of AD stratified based on APOE genotype. We have differentiated these cells towards neural lineage to obtain astrocytes to assess intrinsic differences between those derived from AD patients or healthy controls.

Results: We have implemented a serum-free approach and generated neural precursors and astrocytes from all the lines tested. Cells are different at the phenotypic level, suggesting intrinsic differences in neural cells derived from AD patients.

Conclusions: Human pluripotent stem cell-derived methodology can be used to elucidate the pathogenic pathways associated with neurodegeneration and to identify new therapeutic targets susceptible to modulation, contributing to the development of new effective drugs against AD. Acknowledgments: Supported by Instituto de Salud Carlos III of Spain PI18/01557, PI21/00915 (to AG), PI18/01556 (to JV), and CIBERNED (CB06/05/1116 to AG and CB06/05/0094 to JV); by Junta de Andalucia UMA18-FEDERJA-211 (to AG), PY18-RT-2233 (to AG) and US-1262734 (to JV), Consejeria de Salud PI-0276-2018 (to JAGL) and Programa Operativo de Empleo Juvenil SNGJ4-11 to LCP.
Aims: AD represents the most common cause of dementia in elderly manifested with impairments in cognitive and emotional functions as well as sleep disturbances. Although aggregation of Amyloid-β (Aβ) and neurofibrillary tangles are hallmarks of AD neuropathology, Aβ oligomers have recently been considered as the most neurotoxic species leading to cognitive and non-cognitive manifestations. Here, we analyze their effect on hippocampal adult neurogenesis, known to contribute to neuronal plasticity, and on the circadian system that entrains rhythms in behavior and physiology to the 24h solar day.

Methods: tgDimer mice express Aβ dimers in a soluble state without plaques formation or neuroinflammation, therefore, allows to dissect the pure effect of soluble Aβ dimers in AD pathology. Moreover, they show memory deficits, thus, represent a model for early AD. We investigated adult hippocampal neurogenesis using markers for proliferation (BrdU) and neuroblast (DCX) via immunohistochemistry. Integrity of the circadian system was analyzed by rhythmic locomotor activity, recorded using infrared detectors, and expression of the core clock gene Per1 in the suprachiasmatic nucleus (SCN), the central circadian rhythm generator, by quantitative PCR in tgDimer mice.

Results: We found a significant reduction of BrdU+ neural progenitor cells and DCX+ neuroblasts in tgDimer mice as compared to WT mice. Furthermore, tgDimer mice exhibit higher locomotor activity during early light/rest phase, reminiscent of sundowning behavior in AD patients, and increased sensitivity to chronodisruption, in addition to decreased Per1 expression in the SCN.

Conclusions: Our results show that soluble Aβ dimers impair adult hippocampal neurogenesis and affect the circadian system.
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**Aims:** Neurogenesis, significantly reduced in Alzheimer’s Disease (AD), is a potential therapeutic target. Zebrafish, a well-established AD animal model, can successfully regenerate its diseased brain, hence ideal to study neurogenesis. We aimed to study synergistically and non-synergistically altered pathways between humans and zebrafish using large single-cell and bulk RNA-sequencing (scRNAseq; blkRNAseq) experiments from publicly-available and in-house data from AD-control human and Abeta-injected Zebrafish brains.

**Methods:** We compared six scRNAseq zebrafish datasets (N=10), two scRNAseq (n=27) and three blkRNAseq (N=770) human datasets, restricting to orthologous cross-species genes only (N=20,993). We identified main cell-types clusters and differentially expressed genes (DEG) comparing cases and controls within each cluster. Amongst DEGs, enriched KEGG pathways and Gene Ontology terms were identified.

**Results:** 95\% human and zebrafish brain cells could be co-clustered with neuronal clusters enriched with learning, memory, synaptic transmission, and cognition processes. Using top DEG, we observed high pathways concordance between zebrafish and humans in neuronal clusters (ribosome, ER protein processing, oxidative phosphorylation, long-term potentiation) all implicated in AD. Contrary, human astroglial clusters were uniquely enriched in oxidative phosphorylation and AD processes, while zebrafish clusters in retinol and arachidonic acid metabolism, fatty acid degradation, DNA replication, Notch, JAK-STAT and cytokine signalling. BlkRNAseq found 260 genes non-synergistically DEG between zebrafish and humans, again pointing at apoptotic pathways, JAK-STAT and cytokine signalling.
Conclusions: These findings demonstrate that zebrafish and human transcriptomes can be reliably co-clustered to study AD in animal models. Zebrafish’s uniquely altered pathways could highlight specific mechanisms absent in humans, and potential therapeutic targets to enhance neurogenesis.
POSTERS

OCCURRENCE OF MULTIFOCAL MICROINFARCTS EARLY IN ALZHEIMER’S DISEASE MODULATES THE NEURODEGENERATIVE CASCADE IN A SEX-DEPENDENT MANNER

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Aims: Alzheimer’s disease (AD) constitutes the most common cause of dementia. Vascular abnormalities are well-established as a major AD risk factor. Emerging findings indicate that AD onset increases the prevalence of cerebral microinfarcts, which are essentially caused by micro-occlusions. Although the role of micro-occlusions in AD etiology is well established, how multifocal microinfarct occurrence after AD onset affects neurodegeneration remains unclear.

Methods: In this project, we postulate that cerebral multifocal microinfarcts decisively influence AD pathobiology and progression. Using a novel mouse model of cerebral multifocal microinfarcts associated with micro-occlusions in conjunction with molecular, cellular, imaging, and neurobehavioral approaches, we investigated impact of microinfarct early occurrence in young APP/PS1 mice on AD-like pathology dependently upon biological sex.

Results: Our findings indicate that microinfarct induction in young APP\textsubscript{1}/PS1 mice exacerbates cognitive decline and impairs neurovascular coupling in males, whereas in females those deficits were transient. Microinfarct induction unexpectedly attenuated amyloid-β (Aβ) pathology in males and females and triggered early neuronal loss followed by a robust microglial activation and recruitment of peripheral phagocytic immune cells, more potently in females. Finally, we found out that Dickkopf-1 (DKK1), which exacerbates AD progression, is strongly induced at the lesion sites of males compared to females upon microinfarct induction.

Conclusions: Our study suggests that multifocal microinfarcts aggravate AD pathology more potently in males compared to females in Aβ independent manner via modulation of neurovascular coupling, inflammatory response, and DKK1 expression. Our data indicate that these effects should be taken into consideration when developing accurate AD prognosis tools and therapies.
Aims: Mid-life hypertension is a modifiable risk factor for developing late-life dementia. Anti-hypertensive drugs facilitate the normalization of blood pressure without significantly reducing dementia risk suggesting that some brain damage sustained during these transient hypertensive events are irreversible. The specific brain regions affected as well as how this damage influences cognitive functions remain unclear. We characterized a rat model of transient hypertension with an extended period of normotensive recovery to differentiate recoverable and irreversible damages to the neuronal network.

Methods: F344 rats were treated with L-NG-Nitroarginine methyl ester for one month to induce hypertension. Barnes maze was conducted to assess cognitive functions after one month and four months of normotensive recovery. Immunohistochemistry and electrophysiology were used to examine changes to vascular and neuronal structure as well as to neuronal network functions.

Results: Transient hypertension induced cognitive impairment in rats recovered from hypertension: While deficits in spatial memory recovered, loss of cognitive flexibility persisted. Structurally, in both prefrontal cortex and hippocampus, particularly the former, transient hypertension induced irreversible damages including decreased densities of blood vessels, myelin, and neurons. Corroboratively, electrophysiological analyses showed a compromised network function in both brain regions in addition to increased baseline connectivity between these two regions.

Conclusions: Our results demonstrate that network function between prefrontal cortex-hippocampus is a vulnerable region to hypertension even with intervention and recovery. We provide a mechanistic link between the long-term effects of transient hypertension on dementia risk.
MEDIN, THE MOST COMMON HUMAN AMYLOID, MAY CONTRIBUTE TO CEREBRAL B-AMYLOID ANGIOPATHY

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Aims: Medin (an internal fragment of the protein MFG-E8) aggregates in large arteries of nearly everyone above 50 years of age and was recently proposed as a potential vascular risk factor for dementia. In our study we investigated a potential interaction between medin and Aβ and how it may affect pathology and cognitive decline in Alzheimer’s disease (AD).

Methods: We previously showed that medin aggregates deposit in wildtype mouse brain and cause cerebrovascular dysfunction. Using immunohistochemical and biochemical methods we now investigated whether genetic deletion or exogenous addition of medin can alter Aβ aggregation in vitro and in vivo. To establish translational relevance, we also assessed the transcriptional and protein profile of post-mortem human brain tissue.

Results: Our study provides first evidence for a direct amyloid-amyloid interaction of medin and Aβ. Medin did not only co-localize with Aβ deposits, but genetic deletion or exogenous addition of medin altered onset and neuropathology in two mouse models for cerebral β-amyloidosis. In line with its vascular localization in humans, medin deficiency also reduced cerebral β-amyloid angiopathy and related vascular damage. Notably, protein levels of MFG-E8 correlated positively with β-amyloid angiopathy in APP23 mice. Furthermore, expression of MFG-E8 was significantly increased in AD compared to non-demented patients of the ROSMAP cohort and predicted cognitive decline independent of amyloid pathology.

Conclusions: Our results indicate a new mechanism that may drive age-associated vascular disease and cerebral β-amyloid angiopathy, highlighting medin as a potential therapeutic target to maintain vascular health and cognitive function with age.
CARBONIC ANHYDRASES: UNVEILING NOVEL TARGETS TO RESCUE COGNITIVE DYSFUNCTION, AMELIORATING AB-INITIATED NEUROVASCULAR PATHOLOGY, IN A CEREBRAL AMYLOID ANGIOPATHY (CAA) MOUSE MODEL.

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Aims: To confirm and further dissect the involvement of carbonic anhydrases (CAs) in Aβ-mediated cerebrovascular and neurovascular dysfunction in CAA and AD.

Methods: We employed TgSwDI mice (expressing human Amyloid Precursor Protein, hAPP, carrying the Swedish, Dutch and Iowa mutations), which develop Aβ burden primarily in the cerebral vasculature (starting at 6 months of age), and we fed them a CAI-diet (from 8 to 16 months of age), following which, we performed behavioral analysis, and harvested the brains for both biochemical and immunohistochemical examination.

Results: ATZ- and MTZ-diet improved behavioral performances in the Barnes maze test. CAIs decreased cerebral Aβ ThioflavinS positive deposits, Aβ soluble and insoluble fractions detected by ELISA, along with brain caspase-3 activation. In particular, IHC analysis revealed that CAIs reduce Aβ accumulation in endothelial cells (ECs), astrocytes and microglia, and decrease cell-specific Aβ-induced caspase-3 activity. CAI-treated mice had wider blood vessels and less microhemorrhages compared to untreated Tg mice, indicating improved vascular health. ATZ and MTZ promoted an anti-inflammatory phenotype in both astrocytes and microglia, and induced the expression of TREM2 in microglia, and CD68 in perivascular macrophages, suggesting that enhanced Aβ phagocytosis and degradation may underlie the observed reduction in Aβ aggregates. Furthermore, our results suggested that, in ECs, the CA-VA isoform specifically mediates Aβ-initiated cell death.

Conclusions: Our results showed that CAIs foster endothelial and glial proper function in TgSwDI mice, likely by improving Aβ clearance, and ultimately providing neuroprotection. This work points to CAs as new therapeutic targets for Aβ-induced neurovascular pathology in CAA and AD.
GENERATION OF BLOOD-BRAIN BARRIER MODEL FOR PRECLINICAL DRUG DISCOVERY USING CRYOPRESERVED HUMAN IPSC-DERIVED BRAIN MICROVASCULAR ENDOTHELIAL CELLS, PERICYTES AND ASTROCYTES

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Aims: In vitro models of the Blood Brain Barrier (BBB) include immortalized primary brain endothelial cell lines (BMECs) and pericytes and astrocytes of human and animal origin. These xenogeneic systems do not recreate the physiological restriction of BBB transport in vitro. Isogenic BMECs, pericytes and astrocytes derived from human induced pluripotent stem cells (iPSCs), generated in large quantities, offer an alternative to develop in vitro BBB assays for studying drug permeability and the status of BBB in neurogenerative diseases.

Methods: Episomally-derived iPSCs from apparently healthy normal (AHN) donors and donors harboring the APOE4/4 genotype were successfully differentiated to end-stage cryopreserved BMECs and pericytes using novel defined differentiation protocols. Cryopreserved iPSC-derived BMECs, pericytes and astrocytes were thawed to develop a sandwich BBB model that supported the survival of all three cell types and demonstrated cellular function.

Results: Cryopreserved BMECs post thaw expressed high levels CD31, Glut-1, P-glycoprotein, MRP-1, Claudin -5, ZO-1 and transferrin receptor as detected by immunohistochemical staining and flow cytometry analysis. Cryopreserved BMECs displayed membrane integrity and transport function. Cryopreserved pericytes expressed >90% PGDFR-β+/NG2+/CD13+ along with the co-expression of Desmin+/DLK1+/αSMA+ associated with a contractile arteriolar subtype of pericytes exhibiting phagocytic function. iCell® Astrocytes expressed >90% CD44+/S100β+/GFAP+ and showed glutamate uptake function. Cryopreserved BMECs exhibited TEER values > 2000 Ohm/cm² for up to seven days post thaw. Sandwich BBB models generated with cryopreserved BMECs and pericytes revealed enhanced TEER function >3000 Ohm/cm².

Conclusions: iPSC-derived cryopreserved BMECs, pericytes, and astrocytes enabled the generation of tri-culture BBB models for use in the study of drug delivery and neurodegeneration.
Aims: Metabolic syndrome is a combination of obesity, hyperlipidemia, and insulin resistance and affects a quarter of the worldwide population. It is a major risk factor for development of dementia. Here, we aim to establish a hamster model for brain damage in metabolic syndrome due to the striking similarity between hamsters and humans with regard to cardiovascular and metabolic functions.

Methods: 6-8 week old Golden Syrian hamsters received either a regular diet or cafeteria diet for twelve weeks. Blood sugar and lipid profiles were measured to confirm metabolic syndrome. Dextran extravasation, immunochemistry and immunoblotting were used to examine changes in blood-brain barrier permeability, vessel structure, and neuronal integrity.

Results: In both the prefrontal cortex and hippocampus, two brain regions implicated with cognitive impairment in dementia, cafeteria diet compromised blood-brain barrier permeability as shown by increased dextran extravasation, increased aquaporin 4 expression, and abnormal expression of tight junction proteins. Blood-brain barrier compromise was accompanied by lowered density of cerebral vessels. Furthermore, cafeteria diet induced loss of both NeuN-positive neurons and Iba-1-positive microglia.

Conclusions: Collectively the results demonstrate that we have established a hamster model for metabolic syndrome exhibiting compromise to the cerebrovasculature and brain parenchyma similar to that observed in clinical dementia. This model will help to elucidate the mechanisms underlying the interaction between metabolic syndrome and dementia.
METABOLIC SYNDROME EXACERBATES NEURONAL LOSS AND COGNITIVE IMPAIRMENT IN A RAT MODEL OF ALZHEIMER’S DISEASE

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Aims: Metabolic Syndrome encompasses a group of interconnected metabolic disorders which are associated with a heightened risk for vascular and neurodegenerative diseases including Alzheimer’s disease (AD). Rising evidence suggests that metabolic syndrome is a contributing force in cognitive decline and the deterioration of the brain seen in dementia. This study aimed to investigate the effects of metabolic syndrome on AD using F344TgAD rats with diet induced metabolic syndrome.

Methods: F344TgAD and non-transgenic littermate rats received either a cafeteria diet or a standard chow for twelve weeks. Body weight and food intake were measured weekly, baseline and fasting glucose and a lipid profile was conducted. We used Barnes maze task to assess spatial learning and memory impairments and immunochemistry to examine changes in AD pathology, vessel structure, and neuronal integrity.

Results: The lipid profile revealed that the fatty acid profile, triglycerides, and cholesterol, was changed in the cafeteria diet- fed rats in comparison to the standard diet. In both the prefrontal cortex and hippocampus, cafeteria diet increased expression of aquaporin 4 and decreased lectin density demonstrating compromise of the cerebrovasculature. Cafeteria diet also advanced AD pathology as plaques of amyloid-beta peptides increased along with loss of Iba-1-positive microglia. Cafeteria diet drove metabolic disturbances in the brain which induced neural loss and led to the deficit seen in Barnes maze task.

Conclusions: These results demonstrate that diet induced metabolic syndrome can drive cognitive deficits and degradation of the brain in AD.
DIFFERENTIAL EFFECTS OF KETONE-MODULATING DIETS ON SYSTEMIC METABOLIC ABNORMALITIES IN AN ALZHEIMER'S DISEASE MOUSE MODEL

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Aims: Lifestyle factors contribute to 40% of dementia risk(1). Thus, lifestyle-based treatments appear to be a promising way to counteract AD and dementia. Clinical studies have demonstrated beneficial effects of ketogenic diets on cognitive impairments during aging(2). Here, we investigated the effects of such diets on peripheral metabolic parameters in wildtype and AD mice.

Methods: Young adult 3xTg-AD and B6;129 control mice were administered a control diet (CD, 70% carbohydrate, 20% fat, 10% protein), a CD supplemented with a ketone-yielding substrate (medium chain triglycerides, kMCT diet), or a pure carbohydrate-free ketogenic diet (KD). For 6 months, mice were submitted to a variety of longitudinal (blood samplings, EchoMRI, CLAMS metabolic cages) and terminal (tissue analyses, gene expression) measures of peripheral energy metabolism.

Results: 3xTg-AD and B6;129 control mice on a CD exhibited baseline differences in lipid and glucose metabolism, and differed significantly in their metabolic responses to ketogenic dietary challenges. Interestingly, the kMCT and KD diets also altered metabolic parameters in different ways: the kMCT diet improved systemic metabolic parameters without any sustained increase in circulating ketone levels, while the pure KD diet drove a strong increase in circulating ketones, yet had no obvious beneficial effects on the systemic metabolism parameters we measured.

Conclusions: Our data suggest that familial AD mutations can alter the systemic response to dietary challenges. Furthermore, they support the beneficial metabolic effects of the kMCT diet, and suggest its systemic benefits may operate in part via ketone-independent mechanisms.
Aims: We assessed the effects of empagliflozin (EMP) and antidiabetic polypill (PP) on amyloid pathology, tau phosphorylation and oxidative stress in an Alzheimer’s disease (AD) and type 2 diabetes (T2D) mouse models, as well as in a mixed murine model of AD and T2D.

Methods: Control littermates, AD (APP/PS1), T2D (db/db) and AD-T2D (APP/PS1Xdb/db) mice were treated with EMP (10mg/kg/day) or PP (metformin 200 mg/kg/day, simvastatin 40 mg/kg/day, aspirin 5 mg/kg/day and perindopril 0.5 mg/kg/day) from 1 to 7 months of age. We analyzed amyloid plaques by 4G8 and thioflavin S staining. We also measured soluble and insoluble amyloid-β (Aβ) levels and tau phosphorylation in the cortex of these mice. Cortical lipid peroxidation, by malondialdehyde content, and DNA oxidation, by 8-hydroxy-2'-deoxyguanosine levels, were also assessed.

Results: Amyloid plaque burden was reduced by EMP or PP treatment in APP/PS1 mice, whereas the effect in APP/PS1Xdb/db mice was limited. Cortical soluble and insoluble Aβ40 levels were reduced in APP/PS1 and APP/PS1Xdb/db mice after EMP and PP treatments. Additionally, tau phosphorylation was reduced in diabetic (db/db and APP/PS1Xdb/db). While lipid peroxidation was ameliorated by antidiabetic drugs, differences did not reach statistical significance. Nevertheless, DNA oxidation was counterbalanced in APP/PS1 and APP/PS1Xdb/db mice after EMP and PP treatments.

Conclusions: Overall, long-term EMP and PP treatments improved amyloid and tau pathologies. Antidiabetic treatments also had a positive effect in oxidative stress, supporting the beneficial role of EMP and PP in AD (APP/PS1) and AD-T2D (APP/PS1Xdb/db) mice.
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POSTERS

PREDIABETES, DIABETES AND ALZHEIMER’S DISEASE: AMYLOID DEPOSITION, OXIDATIVE STRESS AND MATRIX METALLOPROTEINASES ACTIVATION IN VIVO AND IN REAL-TIME

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Aims: We analyzed amyloid plaques deposition as well as the presence of reactive oxygen species (ROS) and extracellular matrix metalloproteinases (MMPs) activity associated with amyloid plaques, in vivo and in real time by multiphoton microscopy, in a mouse model of Alzheimer’s disease (AD) and prediabetes together with a mouse model of AD and type 2 diabetes (T2D).

Methods: Prediabetes was induced in APP/PS1 mice by feeding them a high-fat diet for 28 weeks. AD-T2D (APP/PS1xdb/db) mice were generated by crossing APP/PS1 and db/db mice. We performed cranial windows and chronically followed amyloid deposition as amyloid plaques up to 32 weeks of age using methoxy XO-4 and multiphoton microscopy. We also analyzed the presence of ROS by Amplex Red® oxidation and MMPs activity by DQ™ Gelatin in vivo and in real time by multiphoton microscopy.

Results: Amyloid plaque burden is increased in prediabetes-AD mice, without affecting plaque size, while amyloid plaque burden is reduced in AD-T2D mice. On the other hand, MMP activation is increased in association with amyloid plaques in prediabetes-AD mice. Similarly, oxidative stress is increased in prediabetes-AD mice and this effect is more severe when amyloid plaques are analyzed in T2D-AD animals.

Conclusions: Our data show that prediabetes accelerates amyloid plaque deposition, suggesting that prediabetes and overt T2D might not necessarily induce the same phenotypic changes. Also, prediabetes and T2D have a major impact on oxidative stress associated with amyloid plaques, supporting a synergistic effect between AD and metabolic disorders at functional level.
Aims: In the last years, the number of patients suffering from Alzheimer’s disease (AD) has been growing. Evidence suggests that AD is linked with metabolic impairment and brain insulin resistance, but limited studies investigated the effect of diet on the pathology. On the other hand, modern society encourages the consumption of hypercaloric diets, that promote metabolic impairments and result in neuroinflammation and brain insulin resistance, causing the development of several types of dementia.

Methods: Taking in consideration this, the first aim of our experiment was to analyse both the impact of 10-weeks consumption of western diet (20% fat) or mouse chow (7% fat) in wild type and 5xFAD transgenic mice, a model of AD produced by amyloidosis. We measured energy expenditure, peripheral metabolism, plasma concentrations of hormones regulating glucose homeostasis. In the brain, we analyzed the expression of insulin signalling-related proteins and tau and beta amyloid, two proteins linked with AD pathology. These studies were carried in hippocampus and prefrontal cortex, brain regions involved in the development of the pathology and in emotional behaviour. We completed the study by investigating if diet exposure could cause mood alterations like anhedonia and anxiety-like behaviour.

Results: Data indicate that diet resulted in altered behavioural patterns, differences in energy expenditure, respiratory quotient and protein expression in the brain.

Conclusions: Strikingly, wildtype mice exposed to western diets had heavier worsening of those variables, already affected by the genotype, suggesting real damage derived of the consumption of western diets, in absence of a genetic load favouring the development of AD.
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**Aims:** Present study has been designed to elucidate the anti-oxidative, hypocholesterolemic and AD ameliorating properties of the selected edible-medicinal mushrooms: H. erinaceus, L. edodes, F. velutipes and G. lucidum.

**Methods:** Anti-oxidative potential of the mushroom extracts testing; Memory and learning related behavioral testing, Proteomics of the AD rat brains, protein-protein interaction and integrated pathway analyses.

**Results:** As derangement of memory and learning abilities is the most notable complication associated with AD, the effect of the HWE of G. lucidum had been tested upon the AD model rats. Feeding of HWE of G. lucidum was found to improve memory and learning abilities of the AD rats. This cognitive improvement has been supported by elevated levels of memory related neurotransmitters in the respective rats. Transmission electron microscopic (TEM) studies demonstrated pronged neuronal dendrites in the G. lucidum HWE treated rats than those of the non-treated. Finally, brain comparative proteomics have identified differentially expressed proteins involved in neurotransmission, metabolism, cellular stress response and misfolding repairment. Improved functional network had been observed among the proteome of the G. lucidum HWE treated rats through STRING analysis. Ingenuity pathway analysis (IPA) identified nervous system development, cell-cell signaling and interaction, molecular transport and cell death and survival among the top-most functional networks among the experimental subjects.

**Conclusions:** Thus, AD ameliorating effect of G. lucidum through anti-oxidative and hypocholesterolemic performances might be implicated in AD therapeutics.
PERINEURONAL NET FORMATION DURING A TRANSIENT STAY IN AN ENRICHED HOUSING IS NECESSARY FOR COGNITIVE IMPROVEMENTS OF THE TG2576 MOUSE MODEL OF ALZHEIMER'S DISEASE

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Aims: We recently established a link between impaired function of the inhibitory neurons expressing the parvalbumin protein (PV), and cognitive impairments in Alzheimer's disease. PV cells are associated with perineuronal net (PNN), an extracellular matrix appearing at the end of neuronal maturation, the presence of which is reduced in the hippocampus of the Tg2576 mouse (AD mice), perhaps contributing to memory deficits. Importantly, exposure to enriched environment (EE), which has proven long-lasting beneficial effects on memory in AD, also rescues PV/PNN deficits. Here, we hypothesize that EE-induced cognitive improvements in Tg2576 mice are supported by a remodeling of hippocampal PV/PNN cell network.

Methods: We injected chondroitinase-ABC (ChABC), a PNN-degrading enzyme, into the area CA1 of 5-month-old Tg2576 and non-transgenic mice, the day before EE for 10 days. Doing so, we were preventing EE-induced PNN formation in the area CA1, specifically. Twenty days later, the mice were subjected to the object location task (CA1), and social memory (CA2). This allowed us evaluating the link between absence of PNN and memory performance in a specific task.

Results: Here we show that a 10-day stay in EE is sufficient to induce memory improvements of Tg2576 mice. Importantly, blocking EE-induced PNN in area CA1 of AD mice prevented spatial memory improvement that depends on this area.

Conclusions: Increased PNN around PV cells is necessary for the long-lasting beneficial effects of EE on cognitive functions of AD mice. This indicates that strategies aiming at enhancing PV cell function through their PNNs may be alternative therapeutic targets in AD.
Aims: Alzheimer's disease (AD) is a multifactorial disease with a strong genetic background. Recent genome-wide association studies have identified several loci linked to an increased risk of AD. However, genes regulated by these variants and the pathophysiological mechanisms regulated by those genes remain largely elusive. Through this work, we study the role of Bridging Integrator 1 (BIN1), the second most important AD risk gene after APOE, in neurons generated from human induced pluripotent stem cells (hiPSCs).

Methods: hiPSCs which are heterozygous and null for BIN1 were used for our studies along with wild-type control cells. Cerebral organoids were derived from these hiPSCs. Cells or tissues were subjected to immunostaining, calcium imaging, and single nucleus RNA sequencing (snRNA-seq).

Results: We show that deletion of BIN1 is sufficient to cause neuronal hyperactivation and Tau hyperphosphorylation, without changing APP processing towards amyloid-beta production. We also show that BIN1 is mostly expressed in hiPSC-derived glutamatergic neurons and that these cells express genetic signatures coupled with previous exposure to sustained electrical activity both in BIN1 heterozygous and knockout cerebral organoids.

Conclusions: Our results reveal a role for BIN1 in the regulation of neuronal activity in humans and suggest that its implication in AD pathogenesis could be related to the neuronal hyperactivation and network dysfunctions observed in the AD brain.
THE ELECTROPHYSIOLOGICAL SIGNATURE OF THE EARLY- AND LATE-STAGE ALZHEIMER’S DISEASE BRAIN

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Aims: Alzheimer’s disease (AD) is a neurodegenerative disorder characterized by amyloid-β deposition and neurofibrillary tangle formation. Neurodegeneration is frequently described in regions of the cortex (CTX) and hippocampus (HPC) associated with memory formation and processing. Consequently, neurodegeneration results in memory loss as the predominant clinical manifestation of AD. We previously showed that the neuronal dysfunction can anticipate the onset of cognitive symptoms as indicated by the impairment of gamma-band amplitude modulation by the theta-band phase, both in the HPC and in the medial prefrontal CTX in early-stage AD transgenic rats (TgF344-AD). How these electrophysiological alterations progress during the late stages of AD, i.e. concomitant with clinical symptoms, is the objective of this study.

Methods: Extracellular recordings are performed in anesthetized TgF344-AD rats upon lowering of a linear electrode array into the brain areas of interest. Current stimulation in the entorhinal CTX elicited hippocampal evoked and inter-stimulation responses. Local field potentials and the high-frequency component (spiking activity) are filtered and analyzed independently.

Results: In-depth analysis of local field potentials and spiking activity in the HPC neurons upon entorhinal CTX stimulation in late-stage AD TgF344-AD revealed that the electrophysiological signature of the hippocampus is altered in late-stage AD brains, indicating that different neuronal populations are likely affected throughout the course of the disease.

Conclusions: Understanding the progress of the brain network dysfunction in AD in susceptible cortical and subcortical areas, not only serves as a sensitive readout for the pathology but will help to direct and consolidate future treatments, targeting specific neuronal subpopulations.
INTEGRITY OF THREE LARGE-SCALE BRAIN NETWORKS (DMN, SAL, CEN) IN THE CONTEXT OF SUBJECTIVE COGNITIVE DECLINE: A RS-FMRI STUDY.

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Aims: Subjective cognitive decline (SCD) has been proposed as an intermediate diagnostic entity in the continuum between normative decline and the mild cognitive impairment linked to dementia. Consequently, the aim of this work was to explore the changes in resting-state functional brain connectivity, linked to the SCD, of three closely related brain networks (Default mode network -DMN-, Salience Network -SAL-, Central Executive Network –CEN-).

Methods: The sample was composed of 95 participants, comprising a control group of 48 (33 ♀/15 ♂) participants and a SCD group of 47 (33 ♀/14 ♂) participants. An independent component analysis was carried out using the CONN toolbox.

Results: We observed increased connectivity in the precuneus for the ventral subcomponent of the DMN (vDMN) and in cerebellar (crus I/II) and occipital (occipital fusiform gyrus) regions for the CEN, in the control group compared to the SCD group. These results were negatively associated with scores on the patient’s subjective memory complaints test. Moreover, it was observed that Mini-Mental State Examination (MMSE) scores were positively associated with greater DMN-CEN and CEN-SAL anti-correlations in frontal regions in our sample.

Conclusions: In conclusion, subjective memory complaints are associated with altered intrinsic connectivity of the DMN and CEN, and cognitive status, measured by the MMSE, is related to more robust anti-correlations between DMN-CEN and CEN-SAL. This association between the MMSE scores and the degree of anti-correlation of these three networks may underlie compensatory processes in the context of aging or reflect a pathological phenomenon linked to the aging process.
IMPACT OF COGNITIVE TRAINING IN SOMATOSENSORIAL RESTING STATE NETWORK IN THE TGF344AD RAT MODEL

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Aims: TGF344-AD rat model (TG) shows time-dependent alterations in several resting state networks such as default mode, sensorimotor and somatosensory networks (Tudela et al., 2019). The activation of somatosensory network of 18 months old TG rats was correlated with the behavioral performance in the DNMS test, revealing the importance of this network for a proper cognitive outcome. Our aim was to study the impact of cognitive training during 8 months on somatosensory networks in non-aged wild type (WT) and TG rats.

Methods: Training and DMNS test were performed in WT and TG rats starting at 3 months of age until 11 months, when MRI was acquired. rs-fMRI was acquired in a 7T scanner periodically to evaluate the somatosensorial network. The standard deviation of the time-series (Amplitude) of the component was computed for each subject and the differences between groups were evaluated using Kruskall-Wallis test.

Results: The amplitude, as a measure of the magnitude of the BOLD activity, in the somatosensory network (Figure 1A) was significantly increased in the trained TG group compared to the non-trained TG (p<0.05). This was not observed between the WT groups (Figure 1B). Despite the trend to lower amplitude values in non-trained TG versus non-trained WT, no significant differences between genotypes were observed in any of the groups.
Conclusions: Although no significant differences between genotypes were observed, there was a significant difference between non-trained and trained TG rats, pointing to an impact of cognitive training in the somatosensorial network of TgF344-AD rats, that could compensate for the initial pathology-related alterations in these animals.
EPIGENOMICS AND LIPIDOMICS INTEGRATION IN ALZHEIMER DISEASE: PATHWAYS INVOLVED IN EARLY DISEASE.

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Aims: Alzheimer Disease (AD) is the most prevalent dementia. However, the physiopathological mechanisms involved in its development are unclear. In this sense, a multi-omics approach could provide an advance. Thus, the aim of this work is to integrate epigenomic and lipidomic analysis in order to study pathways involved in AD.

Methods: Epigenomic and lipidomic analysis were carried out in plasma samples from patients with mild cognitive impairment (MCI) due to AD (n=22), and healthy controls (n=5). Then, omics integration between microRNAs (miRNAs) and lipids was performed by Sparse Partial Least Squares (s-PLS) regression and correlation between selected miRNAs and lipids were studied by heatmaps. Then, potential target of the miRNAs implied in lipid pathways were identify by miRNA data bases.

Results: 25 miRNAs and 25 lipids with higher loadings in the sPLS regression were selected. Among lipids selected highlights some phosphatidylethanolamines (PE), lysophosphatidylcholines (LPC), ceramides, phosphatidylcholines (PC), triglycerides (TG) and several long chain fatty acids. Fatty acids showed strong positive correlations with miRNAs (hsa−miR−494−3p, hsa−miR−421, hsa−let−7a−3p, hsa−miR−664a−3p, hsa−miR−450b−5p, hsa−miR−654−5p, hsa−miR−2110, hsa−miR−505−3p, hsa−miR−29a−3p, hsa−miR−19b−3p, hsa−miR−185−5p, hsa−miR−576−5p, hsa−miR−28b−3p, hsa−miR−143−3p, hsa−miR−4433a−3p). These miRNAs regulated genes implied in fatty acids metabolism, as elongation of very long-chain fatty acids (ELOVL), and fatty acid desaturases (FADs).

Conclusions: Integration between lipidomics and epigenomic analysis showed a relationship between AD and lipid metabolism, especially in long chain fatty acids.
WUZI YANZONG PILL, A CLASSICAL HERBAL FORMULA, EXHIBITS THERAPEUTIC POTENTIAL IN TREATING PARKINSON’S DISEASE

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Aims: To determine the therapeutic effect of WYP on PD mice and explore its mechanism

Methods: C57BL/6 mice were randomly divided into normal control, PD control, and WYP groups. The WYP group was orally administrated with WYP for 14 days while the normal and PD groups were treated with saline. The ethology were detected by open field and pole tests. The positive expression of TH was detected by Immunohistochemical method. Western Blot were used to detect the contents of Caspase-9, CHOP, ASK1, p-JNK, Caspase-3 and Caspase-12.

Results: Compared with the model group, WYP reduced the pole climbing and total resting time(P<0.05), enhanced the total distant(P<0.01) and mean speed(P<0.05). TH+ neuron in substantia nigra increased after WYP treatment(P<0.05), while the levels of CHOP(P<0.01), Caspase-9 and Caspase-12 decreased(P<0.05). There was no statistically significant change in protein content of ASK1, p-JNK and Caspase-3 in all the three groups.

Conclusions: WYP can improve the balance and coordination ability and alleviate the pathological changes in PD mice. Its mechanism may be related to inhibit the CHOP and Caspase-12 apoptosis pathways. (Grants: NNSF of China 81102552 and 81703978, the Research Project Supported by Shanxi Scholarship Council(No.2021-QN-03), the Young Scientist Cultivation Program Project, Shanxi University of Chinese Medicine (No.2021PY-QN-03), Central Government Guided Local Funding Projects for Science and Technology Development YDZX2020140001483, Returned Chinese Scholars Technology Activities Preferred Project, Shanxi Province of China 20200026, Natural Science Foundation of Shanxi Province 201901D111334, Key Research and Development Projects of Shanxi Province 201803D31209, Shanxi University Science and Technology Innovation Project 2019L0724.)
THE CONTRIBUTION OF HYPERHOMOCYSTENEMIA AND HYPOPERFUSION TO AMYLOID-BETA INDUCED TRAIL DEATH RECEPTOR-MEDIATED CEREBRAL ENDOTHELIAL CELL APOPTOSIS

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Aims: Cerebrovascular dysfunction has been implicated as a major contributor to Alzheimer’s Disease (AD) pathology, with particularly endothelial cell (EC) stress promoting the focal ischemia, cerebral blood flow impairments, and blood brain barrier permeability that are pathologically characteristic in AD. Recent evidence has emerged suggesting a link between cardiovascular (CV) diseases and AD pathology, particularly showing that CV/cerebrovascular risk factors, including hyperhomocysteinemia (Hhcy) and hypoperfusion (oxygen/glucose deprivation (OGD)), contribute to AD pathology and risk. Despite this, the underlying molecular mechanisms for this interaction remain unclear. Previously our lab has demonstrated that amyloid-beta (Aβ), particularly Aβ40-Q22 (vasculotropic Dutch mutant), promotes TRAIL death receptor (DR)-mediated apoptosis in human cerebral ECs. We tested the hypothesis that Hhcy and hypoperfusion exacerbate Aβ-induced cerebral EC TRAIL DR-mediated apoptosis and associated EC stress mechanisms.

Methods: Human cerebral microvascular ECs were challenged with AβQ22 and/or homocysteine (Hcy) in the presence/absence of hypoperfusion. Apoptotic mediators expression, caspase activation, and DNA fragmentation were measured.

Results: AβQ22 and Hcy challenge independently upregulated EC expression of TRAIL DR-related apoptotic mediators and caspase activity, and, at certain time-points, resulted in an additive upregulation of apoptotic mediators and caspase activity. AβQ22, Hcy, and hypoperfusion individually increased DNA fragmentation within cerebral ECs. Combination treatments of AβQ22 and Hcy created an additive effect on DNA fragmentation.

Conclusions: The presence of CV risk factors seems to exacerbate Aβ induced-EC death through the TRAIL DR-mediated apoptotic pathway, revealing one of the specific molecular mechanism through which amyloidosis and CV risk factors may synergistically act to produce EC stress/death in AD pathology.
COGNITION IMPAIRMENT AND NEURODEGENERATION OF 5xFAD MICE ARE AMELIORATED BY FERROPTOSIS REGULATOR GLUTATHIONE PEROXIDASE 4

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Aims: Oxidative damage such as lipid peroxidation is well-demonstrated in Alzheimer’s disease (AD). Lipid peroxidation is the driver of ferroptosis, an iron-dependent oxidative mode of cell death. However, the importance of ferroptosis in AD remains unclear.

Methods: 5xFAD mouse is a widely used AD mouse model, while glutathione peroxidase 4 (GPX4) plays a central role in the regulation of ferroptosis by directly reducing phospholipid hydroperoxides in membranes. To determine the importance of ferroptosis in AD, we generated 5xFAD mice with overexpression of GPX4, i.e., 5xFAD/GPX4 mice, and compared cognition and neurodegeneration between control 5xFAD mice and 5xFAD/GPX4 mice.

Results: Our results indicate that, hippocampal neurons from 5xFAD/GPX4 mice showed increased capacity to cope with lipid peroxidation. Importantly, compared with control 5xFAD mice, 5xFAD/GPX4 mice showed significantly improved learning and memory. 5xFAD/GPX4 mice also exhibited attenuated neurodegeneration and decreased markers of ferroptosis. 5xFAD/GPX4 mice further showed improved locomotor function and reduced weight loss at an advanced age.

Conclusions: Our study demonstrated that enhanced defense against ferroptosis afforded by Gpx4 overexpression attenuated neurodegeneration and ameliorated cognitive impairment in 5xFAD mice. The present findings suggest that ferroptosis plays a key role in AD pathogenesis.
ACTIVATING TRANSCRIPTION FACTOR-4 MEDIATED INHIBITION OF MTOR SIGNALLING RESULTS IN PUMA-DEPENDENT NEURONAL APOPTOSIS IN MODELS OF PARKINSON'S DISEASE

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Aims: Previously we have demonstrated that ATF4 is upregulated in dopaminergic neurons following exposure to MPP+, 6-OHDA, or a-syn PFFs. Specifically, we determined that ATF4 induction promotes neuronal death through transcriptional activation of known death genes including pro-apoptotic BH3-only protein PUMA. Despite ATF4 being required for PUMA activation, chromatin immunoprecipitation experiments have revealed that ATF4 does directly activate the PUMA promoter. Given this, the aim of the current study is to characterize the indirect mechanism by which ATF4 activation signals PUMA-dependent neuron loss.

Methods: To address the mechanism by which ATF4 results in PUMA activation we have used primary mouse neurons, adenoviral constructs, and pharmacological approaches. Specifically, to study the function of ATF4 we have derived primary neurons from wildtype and ATF4-deficient littermates and exposed them to PD toxins (MPP+ or 6-OHDA) and preformed alpha-synuclein fibrils. In addition, we have generated adenoviral constructs which enable the expression of ATF4 or ATF4 containing a mutated DNA-binding domain.

Results: We demonstrate that ectopic ATF4 expression results in activation of pro-apoptotic genes, which result in downregulation of mTOR, and subsequent neuronal apoptosis. Importantly, we have determined that ATF4-deficient neurons are resistant to mTOR inhibition and PUMA activation induced by exposure to a-syn PFFs or PD toxins as compared to wild-type littermates.

Conclusions: These results provide a novel signalling mechanism by which ATF4 indirectly promotes PUMA-mediated dopaminergic neuron cell death through suppression of mTOR signalling and supports the potential for targeting the ISR in the development of therapeutics for PD.
Aims: Parkinson’s disease (PD) is motor disorder but may additionally suffer from auditory perceptual disturbances of acoustic signals to impaired speech processing which can have cascading effect on communication and quality of life in PD. The negative consequences are amenable to auditory habilitation. Therefore, evaluation of auditory perceptual disorders to provide auditory habilitation and to monitor effects of treatment is important in this population. Hence, we intend to assess the auditory processing abilities at subcortical level of auditory pathways by comparing the behavioural behavioral and electropgysiological measures in persons with and without PD.

Methods: Simple purposive sampling technique was used to select 70 subjects in the age range of 55 to 65 years (35 persons without Parkinson’s disease and 35 persons with Parkinson’s disease). Behavioral Audiometry and Click evoked Auditory Brainstem Responses (ABR) at 50 dBSL at stimulus rate of 21.1/sec were measured & compared between both the groups.

Results: ABR latency and amplitude analysis showed no significant difference of wave III absolute latency and amplitude and wave III-V interpeak latencies at 50 dBSL. However, there was reduced amplitude and delayed latencies of peak I and V, and interpeak latencies of peak I-V and I-III at 50 dBSL in PD.

Conclusions: The delayed latency and reduced amplitude of ABR waves probably suggest compromised speed of neuronal transmission and magnitude which may be due to altered subcortical structures of auditory pathways in persons with PD. Thus, ABR can be used as a tool to assess and to monitor the treatment effects at peripheral level in PD.
**Aims:** Geriatric population with sensory neural hearing loss (SNHL) exhibit poor speech understanding. The alterations in cochlear and brainstem neuronal structures are attributed for poor speech understanding. The literature reveals that hearing aids can improve speech understanding and suggests that auditory system reorganizes the auditory structures with the use of hearing aid (HA). However, it is unknown how the neurophysiologic auditory mechanisms changes with exposure to amplified sounds. Therefore, the study aims to examine electrophysiological changes in the auditory brainstem in geriatric population after HA usage.

**Methods:** 16 geriatric with average age of 62.5 years, having bilateral moderate SNHL who had no experience of hearing aid use were examined with click-evoked auditory brainstem response (ABR) to determine absolute latency and amplitude & interpeak latencies at threshold and 20dB above threshold. The subjects were recommended monaural (right ear) HA and divided into study group (SG) and control group (CG). The SG used HAs for a period of 4 months whereas the CG did not during this period. Both groups were reexamined with ABR after minimum 4 months of HA use and electrophysiological measures were calculated and compared.

**Results:** The study reports no significant change in the absolute latency and amplitude of ABR. However, wave V mean amplitude at threshold level and at 20dBSL was approximately 100µV larger (statistically significant at P<0.05) in the ear that used hearing aid.

**Conclusions:** HA use can encourage physiological changes at auditory brainstem level in geriatric population that may facilitate acoustic processing for better speech understanding and may prevent auditory function loss.
NEUROPATHOLOGICAL HALLMARKS OF NEURODEGENERATIVE DISEASES DO NOT ASSOCIATE WITH COGNITIVE PERFORMANCE IN CENTENARIANS

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Aims: Hallmarks of neurodegenerative diseases accumulate with age in the brains of non-demented individuals, which has implications for diagnosis and interpretation of the pathological role of these hallmarks in extreme aging. To investigate the separability of pathological hallmarks of Alzheimer’s disease (AD), we assessed AD-related pathology in an age-continuum of AD and non-demented subjects up to extreme aging. Furthermore, we determined to what extent neuropathology loads discriminate between cognitive performance in centenarians.

Methods: NIA-Reagan amyloid phases, Braak neurofibrillary tangle stages, and CERAD neuritic plaque scores were analyzed in an age continuum comprising 849 AD (aged 37-102), 653 non-demented (aged 16-99) and 86 centenarian (aged 100-115) donors. Centenarian brain tissue was additionally scored for the load of TAR DNA-binding protein 43, Lewy bodies and granulovacuolar degeneration and divided in demented (MMSE <=24, n=39) and non-demented (MMSE>24, n=47), based on MMSE assessment 8.8 (±6.97) months prior to donation.

Results: With increasing age-at-death, AD related neuropathology load increased in non-demented individuals, and decreased in AD cases, converging at around 100 years of age. In centenarians, neuritic plaque load associated with cognitive decline (p=0.02), but all other assessed neuropathologies did not associate with cognitive performance.

Conclusions: The ability of the classic AD-related neuropathological hallmarks to distinguish between health and disease decreases with age until at extreme ages. This suggests that: (1) with age, the ability to maintain cognitive health increasingly depends on being resilient against the toxic effects associated with these pathologies; and/or (2) that neuropathological hallmarks accumulated in older individuals may be a harmless consequence of aging.
EPILEPTIFORM EVENTS IN THE TG2576 MOUSE MODEL OF ALZHEIMER’S DISEASE DO NOT WORSEN WITH AGE BUT ARE EXACERBATED DURING REM SLEEP: INVOLVEMENT OF NORADRENERGIC TRANSMISSION

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Aims: Over the past decade, increasing attention has been paid to the link between brain network hypersynchrony and Alzheimer's disease (AD). We have previously shown that epileptiform events occur in Tg2576 mouse models of AD from a very early age. The main objective of this study was to better characterize hypersynchronous network activity in the Tg2576 mouse model and its relationship with ageing and sleep.

Methods: We recorded the EEG and EMG of Tg2576 mice (1.5 to 13 months of age) during 24 hours. In order to understand why interictal spikes are almost absent during wakefulness, we administrated antagonists of noradrenergic and dopaminergic receptors to a subset of mice.

Results: We show that the frequency of interictal spikes is already at its highest level at an early age (6-weeks old) and does not worsen with aging. We observed that interictal spikes occur preferentially during REM sleep and are phase-locked to the theta cycle. Finally, we found that blocking alpha-1 noradrenergic transmission (with prazosin) triggers the occurrence of interictal spikes during wakefulness.

Conclusions: In T2576 mice, epileptiform events are not related to ageing but highly dependent on vigilance stages: completely absent during wakefulness, they appear during slow-wave sleep and are exacerbated during REM-sleep. We propose that noradrenergic tone protects hippocampal networks from epileptiform events during wakefulness, while its decrease during sleep might unmask excitation/inhibition imbalance.
THE ROLE OF BRAIN SENESCENCE AND TELOMERE ATTRITION IN THE NEUROPATHOLOGY OF ALZHEIMER’S DISEASE

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**Aims:** Aging is the leading risk factor for most neurodegenerative diseases, including Alzheimer’s disease (AD). Recent studies have suggested that AD is tightly correlated with the accumulation of senescent cells during pathological aging, likely due to the progressive build-up of cellular insults. As gradual reduction in telomere length is known to play a central role in cellular senescence, our main objective was to evaluate how brain senescence caused by telomere attrition interconnects with brain dysfunction and AD.

**Methods:** The Terc−/− mouse model of telomere attrition was used to characterize brain senescence. Specifically, brain samples and cell-type specific primary cultures (neurons, astrocytes) were obtained to validate classical and novel markers of senescence and discover alterations in key cellular pathways. We then evaluated how senescence affects the onset and progression of AD by crossing our Terc−/− mice with the 5xFAD mouse model of AD and evaluating several Aβ-induced neuropathological features.

**Results:** Using brain tissue and cell-specific cultures from Terc−/− mice, we described the induction of classical and novel senescence markers and the appearance of an altered autophagy function. We also demonstrated that telomere attrition modifies the progression of amyloid-induced pathology in AD, by reducing Aβ plaque formation but increasing neuroinflammation and accelerating neurodegeneration in specific brain regions related to AD pathology.

**Conclusions:** Overall, our experiments indicate that telomere-induced senescence modulates amyloid pathology and neurodegeneration in a mouse model of AD.
POSTERS

REGIONAL COLOCALIZATION OF Aβ AND TAU PATHOLOGIC CHANGES IN ALZHEIMER’S DISEASE AND CONTROL CASES.

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Aims: The neuropathological schemes for Aβ plaques (Thal phases) and neurofibrillary tau tangles (NFT; Braak stages) report that the neocortex is affected by Aβ plaques in early Thal phase 1 but at late stages by NFTs (Braak stage V-VI). The lack of colocalization between these disease hallmarks make it difficult to reconcile the causative pathogenic link between Aβ and tau. Nevertheless, biochemical analysis suggests that discrete alterations in tau and Aβ may proceed the insoluble aggregate formation. This study sought to determine the regional correlations and colocalization between Aβ and tau pathological changes in the AD and controls cases.

Methods: Fixed/frozen samples of frontal grey (GM) and white matter (WM) (Brodmann area 8/9) of 52 post-mortem human cases were probed for Aβ and tau markers in dot-blot and immunohistochemical assays and quantified according to diagnosis (Control cf. AD) and Braak stage.

Results: Unsurprisingly, phosphorylated (AT8, PHF1, CP13) and Aβ (MOAB-2, non-APP- cross-reacting) were elevated in AD compared with Controls. Interestingly, correlative relationships between Braak stage and AT-8 (GM/WM, p<0.01, r>0.5), PHF-1 (GM/WM, p<0.05, r<0.35) and Aβ (GM/WM, p<0.05, r>0.37) were observed in controls (Braak 0-IV) as were correlations between AT-8 and Aβ (p<0.01, r>0.61). Measures of intracellular Aβ and AT-8 via immunohistochemistry will also be presented.

Conclusions: Our data suggest an early regional co-localisation of Aβ and tau pathology in the GM/WM of the frontal cortex. Such prior low level Aβ and tau interactions may prime the region for later gross disease involvement and may inform on future protective strategies.
IDENTIFICATION OF STRUCTURAL DISCONNECTIONS OF WHITE MATTER TRACTS IN ADULTS WITH AMNESTIC MILD COGNITIVE IMPAIRMENT.

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Aims: Amnestic mild cognitive impairment (aMCI) is a clinical syndrome that often progress to Alzheimer’s Disease (AD) dementia, and in which there is an objective memory decline, preserved activities of daily living and absence of dementia. Recent structural magnetic resonance imaging studies employed the total volume of white matter hyperintensities (WMHs) as a global index of structural brain damage in aMCI or AD dementia. However, these studies did not evaluate how the WMHs location may affect the structural connectivity of the white matter (WM) tracts.

Methods: The impact of WHMs in the integrity of WM tracts was compared between 80 cognitively unimpaired (CU) adults (mean age: 68.96 years, SD ± 7.67) and 57 individuals with aMCI (mean age: 69.39 years, SD ± 8.77). WMHs were identified in T2-weighted Fluid Attenuated Inversion Recovery images and then a lesion network mapping analysis was conducted. Two sample t-tests were performed to compare, between both groups, the total volume, the number of WMHs and the tract-level disconnection of WM tracts included in the Human Connectome Project tractography atlas.

Results: There were not significant differences between groups in the volume and the number of WMHs. However, in comparison to CU adults, individuals with aMCI displayed higher structural disconnection in seven projection, five association and six commissural cortical WM pathways.

Conclusions: Individuals with aMCI display structural signs of disconnections in cortical WM tracts that support cognitive functioning. These findings suggest that the WMHs location could be relevant for detecting cognitive deficits in prodromal stages of AD.
Aims: In this study, we investigated whether long-term culture induces senescence and functional alterations in cultured rat astrocytes and their effects on neuronal function. Methods: We used long-term culture method that mimic the physiological aging over time in cultured rat astrocytes. The aging phenotypes were investigated senescence-associated β-galactosidase (SA-β-gal) activity as a cellular senescence marker and the changes in aging-related factors including nucleus size change and senescence associated secretory phenotypes (SASPs). The presence of astrocytic dysfunctions in cell migration, phagocytosis, and mitochondrial function in aged astrocytes were also investigated as well as the effects of aged astrocytes on neuronal functions. Results: The results indicated that long-term cultured astrocytes showed cellular senescence phenotypes including increased SA-β-gal-positive cells associated with increased nuclear size and increased senescence-associated secretory phenotypes (SASP) such as IL-6 and IL-1β. We also observed dysregulation of cellular functions based on wound-healing, neuronal protection, and phagocytosis assays. Finally, mitochondrial dysfunction was noted through the determination of mitochondrial membrane potential using tetramethylrhodamine methyl ester (TMRM) and the measurement of mitochondrial oxygen consumption rate (OCR). Conclusions: These data suggest that long-term cultured astrocytes show aged cell-like phenotypes through mitochondrial dysfunction, immune activation which may have implications in brain aging and neurodegenerative conditions.
POSTERS

GUT-BRAIN AXIS IN RELATION TO AMYLOID-B AGGREGATION

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Aims: Amyloid plaques in Alzheimer’s disease (AD) are associated with inflammation. Recent studies have demonstrated the involvement of gut in central amyloid-beta (Aβ) pathogenesis; still the mechanisms are not well understood. Dysregulation in gut pathophysiology may be involved in promoting chronic inflammation.

Methods: Using Tg2576 AD mice we tested the hypothesis that gut bears the Aβ burden prior to brain. We used pre-symptomatic 6-months old and symptomatic 15-months old Tg2576 compared to their age-matched littermate WT control mice. We study human AD patients gastrointestinal microbiome profiling.

Results: We identified that dysfunction of intestinal epithelial barrier (IEB), dysregulation of absorption, and vascular Aβ deposition in the IEB occur before central Aβ aggregation is detectible. The intestinal dysfunction observed before brain pathology was associated with elevated inflammatory and angiogenic plasma cytokines including IL-9, VEGF, and IP-10. In association with reduced myelin tight junction proteins in the brain of animals with Aβ pathology, we identified reduced levels of systemic vitamin B12 and decrease cubilin, an intestinal B12 transporter, after the development of central Aβ pathology. Lastly, we report Aβ deposition in the intestinal autopsy from AD patients with confirmed central Aβ pathology that is not present in intestinal autopsy from non-AD controls.

Conclusions: Our novel data provide evidence that gut dysfunction occurs in AD and may contribute to its etiology. In addition, human moderate-severe AD patients showed changes in microbiome of gastrointestinal tract and this was associated with some key changes in cytokines of blood plasma obtained from the same patient compared to age matched healthy controls.
Aims: Alzheimer's disease (AD) is a complex disease with both genetic and environmental etiological contributors. The role of the gut and oral microbiota in this disease has recently become an active area of investigation. This study aimed to examine associations between the microbiota and AD in a human cross-sectional cohort.

Methods: Forty-five AD patients and 54 matched controls were recruited in Vancouver, Canada. Fecal and oral samples underwent 16S microbiota sequencing. A wide array of demographic and clinical data were collected. Differences between participant groups were assessed, and associations between microbes and clinical variables were examined within the AD population.

Results: The gut microbiota of AD patients displayed lower diversity relative to controls, although taxonomic differences were sparse. In contrast, the AD oral microbiota displayed higher diversity, with several taxonomic differences relative to controls, including a lower abundance of the families Streptococcaceae and Actinomycetaceae and a higher abundance of Weeksellaceae, among others. The periodontitis-associated oral microbe Porphyromonas gingivalis was 5 times more prevalent among patients. No significant associations between gut or oral microbes and cognition were detected, but several correlations existed between microbes and mood disorders and BMI among patients, including a strong positive correlation between Alphaproteobacteria and depression score.

Conclusions: The gut microbiota of AD patients was not overtly different from controls, although it displayed lower diversity, an overall marker of microbiota health. Conversely, the oral microbiota did display marked taxonomic differences. Cognition was not associated with a microbial signature, but other relevant AD factors including depressive mood and BMI did demonstrate an association.
POSTERS

AN ORGANS-ON-A-CHIP MIMICKING THE GUT-BRAIN AXIS OF ALZHEIMER’S DISEASE

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Aims: Understanding the mechanisms of gut microbiota and gut-brain axis (GBA) has been an important area of research in Alzheimer’s disease (AD). Despite the extensive studies on GBA from animal models to patient conditions in AD, the precise relationship between the brain and gut pathophysiology is still elusive.

Methods: To investigate brain-gut interactions in AD, we developed an organs-on-chip that integrates multiple organs into one microfluidic chamber and overcomes the limitations of animal models (complex physiology). In this chamber, we used human induced pluripotent stem cells (hiPSCs)-derived organoid models with AD genetic background that could recapitulate the pathological features.

Results: We created a brain-vagus nerve-gut organoid model by culturing patient-derived iPSC to investigate the effect of AD lesions in the brain on the gut environment. First, we compared the phenotypic differences between AD patient- and control-derived brain organoids, vagus nerve, and gut organoids, respectively. Next, to evaluate the impact of brain lesions on gut functions, we developed an organs-on-chip device that can connect the brain to gut via vagal motor neurons. Using genetically engineered gut organoid, we also offered a novel therapeutic strategy to tackle AD pathology through gut-targeted intervention.

Conclusions: In this study, we investigated a novel possibility that gut dysbiosis and disturbances in the gut environment are consequences of brain pathology rather than a cause of AD progression. From this perspective, organs-on-a-chip platform mimicking the brain-vagus nerve-gut pathway represented a new challenge for understanding the complex etiology of AD.
UNDERSTANDING THE ROLE OF PLATELETS IN ALZHEIMER’S DISEASE

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Aims: In Alzheimer’s disease (AD) platelets become dysfunctional. However, it remains unclear whether platelet dysfunction in AD is a consequence of the ongoing pathological events or a driver of the disease. To investigate platelet’s contribution to AD pathology, we used an AD transgenic mouse model (i.e. APP Swedish PS1 dE9, APP-PS1) for cellular and molecular characterization of platelets and immune-mediated platelet depletion.

Methods: We assessed the activation status (CD62P expression), ultrastructure and the proteome of blood isolated platelets in 14 months old APP-PS1 mice and wild type (WT) age-matched controls. Further, we induced short-term immune-mediated platelet depletion in APP-PS1 mice (12-13 months old) by intraperitoneal injections of an anti-CD42b antibody.

Results: APP-PS1 mice showed significantly higher percentages of activated platelets in the brain but only a slight, non-significant, higher platelet activation in the bloodstream. Nevertheless, preliminary proteomics data revealed 77 differentially expressed proteins in APP-PS1 blood isolated platelets compared to WT mice. Interestingly, in the APP-PS1 mouse brain, about 20% of the platelets were located extravascularly. Antibody-mediated depletion successfully induced thrombocytopenia (>99%) in APP-PS1 mice for five days. Platelet depleted APP-PS1 mice showed similar hippocampal and cortical amyloid loads to IgG treated mice. However, in depleted animals, microglia phagocytosis was decreased compared to controls.

Conclusions: Platelets might present an altered cellular and molecular profile in AD, with implications to cerebral processes such as neuroinflammation. Even though the mechanisms underlying platelet’s influence in the brain remain unknown, these findings provide a base for future developments.
DIFFERENCES IN PLASMA BIOMARKERS OF AD: THE IMPACT OF ETHNICITY AND DIAGNOSIS

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Aims: The aim of the current study was to investigate the impact of ethnicity on blood-based biomarkers of Alzheimer's.

Methods: The study is a cross-sectional investigation of differences in levels of plasma biomarkers of AD in a multi-ethnic, community-based study of cognitive aging in older adults (N= 1869). The sample was composed of African Americans (AA)(N=236), Mexican Americans (MA)(N= 855) and Non-Hispanic Whites (NHW)(N=778). Plasma levels of Aβ40, Aβ42, Total Tau and NfL were assayed using Simoa technology. Participants were categorized as cognitively normal (CN), mild cognitive impairment (MCI) or dementia (Dem) using a consensus based algorithm. Levels of the plasma biomarkers within groups by diagnosis and across groups by diagnoses were analyzed with ANOVA co-varying age and gender.

Results: A consistent finding was that for NC and MCI diagnoses NHWs had significantly higher levels of Aβ40, Aβ42 and NfL and AAs had the lowest level with MAs falling in the middle. For the dementia diagnosis the groups did not differ on Aβ42 or NfL with NHWs having the highest level of Aβ40 and AAs the lowest. Comparing across diagnoses within each group there was no significant difference in Aβ40 levels for any of the groups. No difference in Aβ42 across diagnoses was found for NHWs or MAs. For all groups NfL levels were significantly higher in MCI and dementia than the NC group.

Conclusions: Ethnicity needs to be taken into account when studying biomarkers. NfL is a robust maker across groups. The cross-sectional data suggests changes in the biomarker profiles as disease progresses.
PYROGLUTAMYLATED AMYLOID BETA IS ASSOCIATED WITH TAU IN ALZHEIMER’S DISEASE – IMPLICATIONS FOR THERAPEUTIC INTERVENTION IN EARLY DISEASE.

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Aims: Alzheimer’s disease (AD) is characterised by accumulations of amyloid beta (Aβ) and tau proteins. Animal models have demonstrated pyroglutamylated Aβ (pAβ) has a functional connection with tau. Previous studies suggest that clearance of pAβ may reduce early-stage tau pathology in transgenic mouse models and studies from our laboratory have shown pAβ is associated with tau burden in human post-mortem brain tissue, and clinical dementia in patients with AD. The aim of this investigation was to determine whether pAβ is associated with immature tau pathology and could be a target for immunotherapy treatment.

Methods: Tissue micro array slides from 60 AD cases and 54 control cases containing 15 brain regions were immunohistochemically stained with pAβ and immature tau markers MC1 and CP13. Percentage area of immunopositivity was determined using an automated microscopy system.

Results: Associations were observed between pAβ and both early tau markers in cortical regions: MC1; frontal $r_s=0.287$, p<0.05, temporal $r_s=0.434$, p<0.01, parietal $r_s=0.374$, p<0.05, and occipital $r_s=0.238$, p<0.05, and CP13; frontal $r_s=0.728$, p<0.05, temporal $r_s=0.636$, p<0.05, parietal $r_s=0.723$, p<0.05, and occipital $r_s=0.276$, p<0.05. No associations were observed in subcortical regions (all p>0.05).

Conclusions: Our data suggests pAβ may be associated with conformational states of tau that represent early stage tau depositions, in regions affected early in the disease process (temporal cortex). Therefore, studies investigating whether immunisation using pAβ is capable of removing early stage tau depositions, precluding excessive mature tau accumulations, and attenuating cognitive decline in AD are warranted.
Aims: The first pathogenic event along the Alzheimer's disease continuum which becomes apparent is the brain deposition of Aβ starting at least 20 years before clinical disease onset. The link between Aβ and downstream events such as tau pathology and neurodegeneration remains unclear – although this knowledge appears crucial at a time when clinical trials are increasingly shifted to pre-symptomatic disease phases. Consequently, the design of preventive treatment studies based on biomarkers is challenging.

Methods: We analysed the trajectories of brain amyloid load, Aβ seeding activity, cerebrospinal fluid (CSF) neurofilament light (NfL, a neural cytoskeleton protein considered as promising biomarker for neurodegeneration), CSF pTau and glia activation in an APP transgenic mouse model. We then experimentally blocked Aβ production through BACE1 inhibition at distinct amyloid disease stages for either short-term or long-term and also analysed APP tg mice on Tau knock-out background. Immunoassays, immunohistochemistry, and a well-established in vivo seeding assay were used.

Results: Inhibiting Aβ generation in amyloid-laden mice reduces amyloid load and associated microglial activation, but fails to alleviate Aβ seeding activity and CSF NfL increases further. When Aβ inhibition is started at pre-amyloid stage, amyloid deposition and Aβ seeding activity could be largely reduced and strikingly, there was no increase of CSF NfL.

Conclusions: Our data indicate an uncoupling of amyloid load and neurodegeneration along disease progression in mice and presumably in humans.
A NOVEL ROLE FOR APP IN NEURAL CIRCUIT FUNCTION: IMPLICATIONS FOR AD THERAPY

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Aims: Recent evidence has implicated amyloid precursor protein (APP)-derived peptides, including amyloid-beta, in abnormal brain activation that precedes cognitive impairment in Alzheimer’s Disease (AD). However, despite the importance of the APP protein family to AD pathophysiology and therapy, how these contribute to normal brain circuit function remains unclear. Here, we sought to interrogate how the APP family regulates neuronal cell function and long-range neural circuit dynamics.

Methods: APP<sup>flox/flox</sup>APLP2<sup>flox/flox</sup>APLP1<sup>−/−</sup> mice were crossed with NexCre mice expressing Cre prenatally from ~E12.5 in postmitotic neuronal precursor cells of the cortex and hippocampus, producing a conditional triple knockout model (cTKO) of the APP family in excitatory forebrain neurons that is associated with pronounced behavioral deficits (Steubler et al., 2021, EMBO Journal). Neuronal function at the single cell/unit and circuit-level was characterised using in-vivo two-photon and mesoscopic calcium imaging, and bilateral Neuropixel recordings in awake and anesthetised animals.

Results: Cortical neuronal activity in cTKO mice was profoundly suppressed relative to controls, with a marked loss of cross-hemispheric coherence of oscillatory activity (including sleep-dependent slow waves). These cellular and circuit-level impairments were tightly correlated and were partially rescued by NMDA receptor agonism. These findings indicate that the APP family plays a critical, but previously unknown, role in modulating neuronal excitability and long-range circuit connectivity in-vivo.

Conclusions: Our research provides novel and important mechanistic insights into the physiological function of the APP family and their potential role in AD pathogenesis, with important implications for current and future AD therapeutic approaches.
Aims: Induced pluripotent stem cell (iPSC)-based AD models enable investigation of pathomechanisms in human, disease-relevant brain cell types and thus offer great potential for mechanistic and translational studies. However, current iPSC-AD models often show low reproducibility and cell type diversity and lack physiological human cell-cell and cell-matrix contacts. In addition, they typically only enable investigation of early pathologies including endosomal abnormalities and Aβ accumulation but lack hallmarks such as widespread protein aggregation and neuroinflammation. Therefore, we aim to develop novel, more reproducible iPSC-AD models made of multiple brain cell types that enable investigation of pathomechanisms in a 3D cortical tissue-like environment.

Methods: To create these models, we generated iPSCs with synergistic AD mutations by genome editing, and optimized protocols to differentiate these iPSCs into disease-relevant brain cell types, including cortical neurons, astrocytes and microglia. By 3D co-culturing all cell types we established highly controllable and reproducible human cortical tissue models.

Results: Our cultures are stable and largely postmitotic, form functional synapses over time, and display a dense network of neurites and astrocytic processes that is stable for more than 1 year and tiled by ramified microglia. Using our AD lines, we observed typical phenotypes such as increased Aβ secretion and pTau levels, extracellular Aβ accumulation, and potential microglial activation. Currently, we are optimizing the model to elicit later-stage pathology such as Aβ plaques and further investigate microglial states and reactivity.

Conclusions: Our model will form the basis for studies elucidating novel, potentially human-specific pathomechanisms and provide a framework for therapeutic screening approaches.
Aims: In clinical practice, some patients with initial dysexecutive amnesia (recognition preserved) evolve to hippocampal amnesia (recognition impaired), this latter associated with Alzheimer’s disease (AD). We aim to evaluate if there are clinical or neuropsychological characteristics that predict such evolution and evaluate if there is an association between those patients with hippocampal amnesia and the diagnosis of Alzheimer’s disease in our cohort.

Methods: An observational cohort study was designed. Patients with dysexecutive amnesia at the first neuropsychological examination and with available neuropsychological follow-up were collected; subsequently, they were classified according to whether there was an evolution to hippocampal amnesia or whether they remained stable. The clinical and neuropsychological characteristics of the first examination were analyzed, and differences between groups were observed.

Results: Ninety-five patients presented with dysexecutive amnesia were collected (mean age 82.68 years, 50.5% were women, mean time between examinations 1.8 years, and mean clinical follow-up 8.55 years). Forty-five of them (47.4%) presented an evolution to hippocampal amnesia in the following explorations (associated with AD diagnosis in our cohort, p-value 0.004). In the multivariate analysis, a learning rate lower than 3 (OR4.3 CI95%1.7-10.8) and the presence of more than two false positives in recognition in a random list (OR3 CI95%1.1-8.2) in the first examination was statistically significantly related to progression to hippocampal amnesia.

Conclusions: In patients with initial dysexecutive amnesia, assessing the learning index and evaluating false positives on a random list helps predict progression to hippocampal amnesia. Moreover, evolution to hippocampal amnesia was associated with Alzheimer’s disease diagnosis.
Aims: Although epilepsies and neurodegenerative disorders show pathophysiological similarities, their direct functional associations are understudied. Here, we tested the hypothesis that experimental seizures are sufficient to induce tau-related and amyloidogenic modifications in the hippocampus over time.

Methods: We used a model of mesial temporal lobe epilepsy (MTLE) where unilateral intra-hippocampal injection of kainic acid (KA) in C57BL/6 mice elicits robust epileptogenesis and spontaneous seizures. As a corollary, we used a model of generalized status epilepticus (SE), without epileptogenesis, obtained by scaling intraperitoneal KA injection in mice. A schedule of 72h, 1- and 8-weeks after KA was used for analyses.

Results: In experimental MTLE, we show tau-hyperphosphorylation during epileptogenesis (72h-1week) and long-term (8weeks) at spontaneous seizures in the hippocampal foci. These changes extended to the contralateral hippocampus, a region characterized by EEG seizures propagation and no histological lesion. In this model, the induction of amyloidogenic pathways was prominent and long-lasting at the epileptic foci. These Alzheimer’s disease (AD) markers reciprocated an enduring glial inflammation and inadequate activation of the principal endogenous anti-inflammatory system (glucocorticoids). By contrast, following a generalized SE and in the absence of epileptogenesis, the activation of amyloidogenic and hyperphosphorylation markers in the hippocampus was time-dependent, with resolving inflammation. Finally, when comparing MTLE mice to a J20 model relevant to AD, we identified specific overlapping profiles of markers long-term.

Conclusions: Experimental MTLE and generalized SE cause persistent or transient tau-related and amyloidogenic modifications in the hippocampus. In MTLE, the AD trajectory is intertwined with neuroinflammation and it extends to a seizure-propagating region.
IDENTIFYING DISEASE MECHANISMS CAUSED BY ENDOCYTIC RISK GENES IN LATE ONSET ALZHEIMER’S DISEASE

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Aims: The endocytic pathway is crucial to the normal functioning of neurons and microglia, two cell types that use it in highly specialised ways. Endocytic risk genes for Alzheimer’s include BIN1 (p = 6.9x10-44), the second most significant LOAD risk factor after APOE, PICALM (p = 9.3x10-26), CD2AP (p = 5.2x10-11) and SORL1 (p = 9.7x10-15). All of these encode proteins that are key to endocytic recycling and clathrin mediated endocytosis (CME). Current evidence suggests endocytic AD SNPs generally cause a reduction in gene expression as a whole or, as is the case for BIN1, of specific brain isoforms. We therefore aim to understand how changes in expression of these genes drives disease mechanisms in human neurons and microglia.

Methods: We have created heterozygous and homozygous knockout iPSC lines for BIN1 and PICALM. Using iPSC-derived neurons and microglia we examine how alterations in endocytic capacity lead to cell specific phenotypes using live imaging, biochemical and immunocytochemical techniques.

Results: We have successfully created multiple BIN1 and PICALM Kolf2 iPSC homozygous and heterozygous knockout lines using a CRISPR/Cas9 approach. We are currently differentiating these lines to both neurons and microglia to examine disease mechanisms.

Conclusions: Endocytic dysfunction is a key feature of Alzheimer’s disease. Understanding how endocytic risk genes can alter the function of neurons and microglia in potentially different ways to drive disease mechanisms is crucial.
Aims: Blood-brain-barrier (BBB) presents a significant challenge for CNS drug delivery. Transient opening of BBB using Focused Ultrasound (FUS) has been shown to enhance delivery of therapeutics to the brain. Here, we aimed to determine the effects of an anti-pyroglutamate-3 amyloid-beta mAb (07/2a) combined with FUS in an AD-like mouse model.

Methods: First, 24 mo-old APP/PS1dE9 mice were i.v. infused with a single dose of 300 μg 07/2a with or without hippocampal FUS sonication (n=5/group) and euthanized 4 or 72 hr later; brain antibody levels were measured by ELISA. Next, 16 mo-old APP/PS1dE9 mice were treated weekly for 3 weeks with PBS (n=9), 500 μg 07/2a alone (n=9), FUS alone (n=7) or 07/2a + FUS combination (n=6). Water T Maze (WTM) was performed 1-2 weeks later followed by euthanasia.

Results: FUS increased 07/2a mAb levels in brain by 5.9-fold 4 hr after treatment (mean=41.7 pg/mg mAb alone vs. 244 pg/mg mAb+FUS, p<0.005) and 5.5-fold 72 hr post-treatment (31.5 pg/mg mAb alone vs. 173 pg/mg mAb+FUS, p<0.05). Immunohistochemistry confirmed a significant increase in IgG2a mAb staining in the mAb+FUS treated mice at 4 hr (p=0.007) and a strong trend at 72 hr compared to mAb alone. Three weekly treatments of 07/2a improved cognition, when combined with FUS, this improvement occurred faster and led to reduced hippocampal plaque load compared to PBS control mice.

Conclusions: FUS treatment increased 07/2a delivery to the brain, enhanced amyloid-beta plaque clearance and improved cognition in aged APP/PS1dE9 mice, suggesting an additive effect by combining treatments.
Aims: Soluble Abeta-oligomers have been found to be highly neurotoxic and are thought to be responsible for onset and progression of Alzheimer’s disease (AD). Therefore, the elimination of Abeta-oligomers is a promising strategy for therapeutic drug development. In former experiments, compounds that eliminate soluble oligomers of recombinant Abeta in vitro also improved cognition in vivo in transgenic AD mice. Using brain homogenate from transgenic mice and human AD patients as a source for native aggregated Abeta within a complex matrix for screening oligomer-eliminating compounds should predict in vivo efficacy with greater accuracy than a purely in vitro approach alone.

Methods: Abeta-assemblies from brain homogenates are separated according to their particle size by density gradient ultracentrifugation. Brain homogenates as well as multimeric Abeta-species enriched in a certain fraction of brain homogenate are treated with oligomer-eliminating compound RD2 and are then analyzed by the sFIDA assay, a highly sensitive method for detecting and quantitating protein oligomers and aggregates.

Results: An RD2 dose- and incubation time-dependent reduction of Abeta-oligomers in brain homogenates treated with RD2 was observed.

Conclusions: The effect of RD2 on Abeta-assemblies from mouse brain homogenate was in accordance with previous in vitro and in vivo observations. Importantly, Abeta-oligomers in human brain homogenates were eliminated by RD2 as well. Our approach is suitable to predict therapeutic efficacy with higher accuracy than the in vitro approach alone before starting large preclinical animal studies, thus potentially reducing the number of animals used for in vivo tests and enhancing the translational value from preclinical studies to clinical trials.
Aims: Cognitively impaired old-aged beagle dogs - a model of sporadic Alzheimer’s disease - were treated with RD2, a novel drug candidate, which led to a significant reduction of cognitive deficits. With the surface-based fluorescence intensity distribution analysis (sFIDA) oligomers and aggregates could be detected and quantified with single particle sensitivity. In this study the concentrations of Aβ and tau oligomers and aggregates in CSF of these dogs were measured before and after treatment with placebo or RD2 to analyse the effect of RD2 on these biomarkers.

Methods: The dogs were treated orally with low dose or high dose of RD2 or placebo for three months. Using the digital sFIDA method, the concentrations of Aβ and tau oligomers and aggregates in the collected CSF samples were determined.

Results: The treatment with RD2 at high dose led to a reduction of the tau aggregate concentration, while in dog samples with RD2 at low dose and in placebo treated group tau aggregates increased significantly during treatment. However, no significant changes in Aβ concentration were detected in the treatment and placebo groups comparing before and after three-month treatment.

Conclusions: sFIDA proves as a useful method to determine Aβ and tau aggregate concentrations in CSF, and to investigate quantitatively the effect of oligomer targeting drugs. Even though no reduction of Aβ oligomers was observed, the reduction of tau oligomers in the high dose group indicates that by targeting Aβ oligomers downstream targets like tau oligomers could therefore also be affected.
AMYSEEDS: TARGETING AMYLOID BETA SEEDS AT THE INITIAL STAGE OF ALZHEIMER’S DISEASE

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Aims: - Identification and isolation of early Aβ seeds from human brain tissue. - Characterization of isolated early Aβ seeds. - Screening of structure-based therapeutic compounds.

Methods: 1) Identification of healthy human brains containing Aβ seeds. Brain samples will be assessed for the presence of Aβ seeds using a new cellular assay [Aoyagi, A. et al., 2019]. 2) Isolation of early Aβ seeds. A phase transition-based method [Kostylev, M.A. et al., 2018] will be used to isolate and concentrate Aβ assemblies under native conditions from human tissue. 3) Biochemical and structural analysis. The composition and aggregation state of the Aβ seeds will be assessed by different methods (e.g., mass spectrometry) and the structure by cryo-electron microscopy. 4) Virtual and high-throughput screening (HTS). Based on the structural and conformational features of the Aβ seeds, a variety of compounds will be selected. Aβ seeds/compounds interaction will be assessed by HTS. 5) In vitro and in vivo validation of the capacity of the selected compounds to block Aβ seeds.

Results: The presence of early Aβ seeds in human brains before the start of deposition has not been confirmed. This project will prove their existence for the first time and will identify structure-based compounds with therapeutic potential. This same approach of blocking early seeds could be adapted to other neurodegenerative diseases in which pathogenic misfolded proteins are involved.

Conclusions: The success of the AMYSEEDS project will represent a great step forward for understanding the start of AD and hopefully will lead to the discovery of a disease-modifying therapy.
LOW-DOSE RADIATION THERAPY EFFICIENTLY IMPROVES MEMORY DEFICITS AND REDUCES KEY HALLMARKS OF ALZHEIMER’S DISEASE IN TGF344-AD RATS.

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**Aims:** Numerous treatments against AD have been developed to target amyloid, tau or neuroinflammation, without a clear success, highlighting the necessity to develop new therapeutic strategies. Radiation therapy (RT), one of the mainstay cancer treatments, has been recently studied in AD. It has been postulated that this treatment, applied at low doses, could achieve two important effects for AD: a decrease of amyloid load and neuroinflammation. Only 5 studies evaluated its impact in the brain of AD mouse models, using different regimens or delay post RT. The main objective of this study was to assess the clinical relevance of a reference regimen in an AD rat model.

**Methods:** TgF344-AD rats, presenting amyloid-related transgenes, were unilaterally treated with 10 Gray, delivered in 5 daily fractions of 2 Gy either at a pre-symptomatic stage (no cognitive alteration, few plaques and low neuroinflammation) or at an advanced stage (high amyloid load and neuroinflammation, with memory deficits). The beneficial outcomes of RT were assessed using behavioral, histological, biochemical and gamma counting measures, 2 or 4 months after treatment.

**Results:** In young animals, we measured a clear decrease of amyloid from poorly aggregated forms [Aβ₄₀ (-52%), Aβ₄₂ (-42%), Aβ oligomers (-54%); p<0.01] to highly aggregated forms [Aβ₄₀ (-81%) and Aβ₄₂ (-60%); p<0.01]. In addition, we observed a restoration of TSPO levels, a classical marker of neuroinflammation, after LD-RT (p<0.001), accompanied by a decrease of cytokines (IL-1α, TNFα, MCP-1, p<0.05). In old rats, LD-RT significantly improves memory.

**Conclusions:** This study showed promising results to propose LD-RT in AD.
INNATE IMMUNITY STIMULATION IN A NON-HUMAN PRIMATE MODEL OF SPORADIC CAA

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Aims: Extensive evidence suggests that dysregulation of innate immunity plays a key role in Alzheimer's disease (AD) pathogenesis. Our recent data from a non-human primate (NHP) model that develops age-associated cerebral amyloid angiopathy (CAA), squirrel monkey (SQM), demonstrate that stimulation of innate immunity via TLR9 agonist, class B CpG ODN, results in CAA reduction without toxicity. We advanced our study using class C CpG ODN, which is currently in clinical trials for multiple indications. This is the first study assessing the effectiveness of immunostimulatory class C CpG in an aged population whose immune system might be senescent.

Methods: Geriatric female SQMs received either CpG ODN or saline every 4 weeks as part of an ongoing chronic-treatment study. Plasma biomarkers of age-associated neurodegeneration and autoantibody responses towards Abeta 40/42 were assessed using Luminex and Simoa technology. Changes in mRNA expression levels were periodically evaluated in isolated PBMCs prior to and 24hrs post-injection using Nanostring nCounter System. T2-w MRI images were acquired to monitor for ARIA-E.

Results: Nanostring analysis demonstrated significant up-regulation of IFN-inducible genes (ISG-54K/IFIT2, Mx2/MxB, GBP1) and specific cytokine-chemokine genes following CpG ODN injection. IgG/IgM autoantibodies against Abeta did not increase longitudinally. Plasma GFAP and NfL levels were reduced over two years, indicating a beneficial effect of our treatment approach. No ARIA-E like signal abnormalities were observed in CpG-ODN treated animals. Cognitive assessments are underway.

Conclusions: The present study provides essential information prior to clinical use of CpG ODN for AD. We confirm that CpG ODN Class C effectively induces immunostimulatory responses without toxicity.
Aims: Evidence suggests that an intermediate state of aggregates, oligomers, are the most potent neurotoxic species. Our lab has demonstrated α-Syn oligomers can form distinct conformations in the presence of different physiological inducers. By utilizing our α-Syn Toxic Conformation Monoclonal Antibodies (SynTCs) in a combination of methods, we investigate the biological relevance of α-Syn oligomeric polymorphisms.

Methods: Recombinant α-Syn oligomers are modified by physiological inducers, dopamine, docosahexaenoic acid, and artificial cerebrospinal fluid (aCSF). Each oligomers preparation was preincubated with a SynTC at a ratio of 1:4 for 30 minutes. Following preincubation, SH-SY5Y cells, primary neurons, and primary astrocytes isolated from human overexpressing α-Syn mice were treated for 24 hours. The cellular effects of oligomer treatment and oligomer immunodepletion were evaluated utilizing immunoblotting, cell-based assays, and immunocytochemistry.

Results: α-Syn oligomers, modified by different physiological inducers, exhibit distinct neurotoxicity, inflammation, and overall presence of α-Syn. The ability of SynTCs to differentially immunodeplete α-Syn oligomers in distinct cellular conditions further supports our hypothesis of α-Syn polymorphs differentially contributing to the neurotoxicity and neurinflammation observed in synucleinopathies.

Conclusions: With evidence showing oligomers as the most toxic species, neuroprotective intervention strategies targeting stable, toxic oligomers could be disease-modifying. These results exhibit the differential properties of α-Syn oligomers and the effects of therapeutically targeting distinct conformations of α-Syn oligomers. Our next step is to conduct an immunotherapy study in a synucleinopathy mouse model. This will evaluate the in vivo physiological effects of α-Syn oligomeric polymorphisms and if their therapeutic targeting can reduce or halt pathological implications and behavioral phenotypes associated with synucleinopathies.
SELECTIVE TARGETING OF NEUROTOXIC AB42 OLIGOMERS BY SINGLE DOMAIN ANTIBODIES

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Aims: Alzheimer’s disease (AD) is the most widespread neurodegenerative disorder marked by the presence of extracellular amyloid β (Aβ) plaques and intracellular tau neurofibrillary tangles in specific brain tissues. Small, soluble oligomers, rather than mature fibrils, are the major neurotoxic agents. The isolation and characterization of these oligomers is very challenging because they are transient, heterogeneous and low concentrated. Single domain Abs (sdAbs), composed only of a variable domain of the heavy chain with high specificity and affinity and a low inherent toxicity, have been proposed as promising tools for the early diagnosis and therapy for AD.

Methods: We performed an in vitro and in vivo screening of different sdAbs, selecting those with highest preferential binding to Aβ42 oligomers with respect to monomers and fibrils, by ELISA, dot-blot and the super resolution stimulated emission depletion (STED) microscopy. We investigated the ability of sdAbs to prevent Aβ42-induced cytotoxicity in neuronal cells. Then, the selected sdAbs were used to detect and quantify the concentration of Aβ42 assemblies in the cerebrospinal fluid (CSF) of AD patients and controls.

Results: sdAbs can detect neurotoxic Aβ assemblies and prevent the associated neuronal dysfunction in our cell models. Furthermore, sdAbs significantly discriminated Aβ aggregates in the CSF.

Conclusions: All these data provide solid grounds for the understanding of the molecular basis of oligomers toxicity, thus establishing a compelling structure-toxicity relationship. Furthermore, our results pointed sdAbs as suitable tools for the development of a new immunodiagnostic test for the early diagnosis and therapy of AD.
Aims: Ubiquitin carboxyterminal hydrolase L1 (UCHL1) is deubiquitinating enzyme and part of the ubiquitin-proteasome system (UPS). We previously found that immunomodulation with glatiramer acetate (GA) increased expression of osteopontin (Spp1) in macrophages infiltrating the brains of GA-immunized transgenic mouse models of AD (ADtg mice). Interestingly, we identified that UCHL1 expression was Spp1-dependent in vitro. The effects of GA on cerebral UCHL1 levels in vivo in ADtg mice are unknown.

Methods: The expression of UCHL1 in GA-immunized ADtg mice and the extent by which GA impacts UCHL1 expression in primary bone marrow-derived macrophage (BMMF) cultures were evaluated.

Results: Proteomics analysis revealed that UCHL1 was the most downregulated protein in Spp1-deficient and most upregulated protein by GA activation in macrophages. We validated this novel observation that macrophages indeed express UCHL1 and its expression is regulated by Spp1, corroborated by co-immunoprecipitation. Further, we confirmed that UCHL1 colocalizes within Spp1-expressing Iba1+CD45high infiltrating monocytes surrounding Aβ plaques in cortices of ADtg mice. Analysis of UCHL1 revealed a 30% loss of UCHL1 expression in pyramidal neurons in the hippocampi and cortices of ADtg mice relative to WT mice. Importantly, we found that GA immunomodulation restored UCHL1 neuronal expression along with reduced Aβ pathology and gliosis in ADtg mice.

Conclusions: UCHL1 is expressed by BMMFs in vitro as well as in vivo in infiltrating myelomonocytes and is dependent on Spp1 regulation. Further, immunomodulation with GA treatment restored UCHL1 expression in the hippocampus and cingulate cortex of ADtg mice and was associated with cognitive preservation.
NOVEL ENGINEERED NANOBODIES SPECIFIC FOR N-TERMINAL REGION OF ALPHA-SYNUCLEIN RECOGNIZE LEWY-BODY PATHOLOGY AND INHIBIT IN-VITRO SEEDED AGGREGATION AND TOXICITY

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Aims: Nanobodies (Nbs), the single-domain antigen-binding fragments of dromedary Heavy-chain antibodies, are excellent candidates as therapeutic and diagnostic tools in synucleinopathies because of their small size, solubility and stability. Here, we developed and characterized nanobodies specific to the monomeric form of alpha-synuclein (alpha-syn).

Methods: We constructed an immune nanobody library specific to monomeric form of alpha-syn. The screening of the library and epitope mapping was done by phage display technology and ELISA respectively. Monovalent Nb alpha-syn01 was engineered into a bivalent format named BivNb alpha-syn01. Binding interaction to alpha-syn was evaluated by vacuum filtration, isothermal titration respectively and circular dichroism spectroscopy. The activity of nanobodies was done by in-vitro seeded aggregation assay and toxicity assay and the immunohistochemistry using nanobodies on post-mortem brain tissue from cingulate gyrus of a PD case. Binding interactions of Nb alpha-syn01 with the peptide and fibrillar forms was done by protein docking.

Results: Nb alpha-syn01 and its bivalent format recognized preferentially alpha-syn fibrils compared to the monomeric form. Nb alpha-syn01 and BivNb alpha-syn01 were also able to inhibit alpha-syn-seeded aggregation in vitro and reduced alpha-syn-seeded aggregation and toxicity in cells showing their potential to reduce alpha-syn pathology. Both nanobody formats were able to recognize Lewy-body pathology in human post-mortem brain tissue from PD and DLB cases. Structural docking showed that Nb alpha-syn01 binds the N-terminal region of the alpha-syn aggregated form.

Conclusions: These results highlight the potential of our Nanobodies as tool to develop a diagnostic or a therapeutic method for PD and related disorders.
**INDUCTION OF AN EFFECTIVE ANTI-AMYLOID-B HUMORAL RESPONSE IN AGED MICE**

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**Aims:** Aging-related decline in immune functions, termed immunosenescence, is a primary cause of reduced vaccine efficiency in the elderly, due to impaired induction of cellular and humoral responses to new antigens, especially if the response is T cell dependent. Consequently, the elderly are susceptible to a more severe morbidity following infections, and exhibit more prolonged and frequent hospitalization, and a higher mortality rate than in the general population. Therefore, there is an increasing need to develop vaccination strategies that overcome immunosenescence, especially for aging-related diseases such as Alzheimer's disease (AD). This study aimed to develop a new vaccination strategy that harnesses memory-based immunity, which is less affected by aging.

**Methods:** Aged 5xFAD mice, which model early onset AD, as well as aged wildtype mice exhibit a dramatic reduction in anti-Amyloid-beta (A-beta) antibody (Ab) production. We therefore aimed to reverse this process by inducing memory response at a young age. To this end, young mice were primed with a DNA vaccination against the vaccine carrier Hepatitis B surface antigen (HBsAg). At an advanced age, primed mice were immunized with an A-beta₁₆₇ fused to HBsAg.

**Results:** This vaccination scheme elicited a markedly higher A-beta specific antibody titer than vaccinating aged unprimed mice with the same construct. Importantly, this vaccine strategy more efficiently reduced cerebral A-beta levels and altered microglial phenotype.

**Conclusions:** Overall, we provide evidence that priming with an exogenous antigen carrier can overcome impaired humoral responses to self-antigens in the elderly, paving the route for a potent immunotherapy to AD.
Aims: Elevated risk of developing Alzheimer’s disease is associated with epigenetic upregulation of iRhom2, which regulates trafficking and enzymatic activity of A Disintegrin and Metalloprotease 17 (ADAM17) in microglia. How increased levels of iRhom2 affect the onset of AD is unknown. To address this question, we identified iRhom2/ADAM17 substrates from microglia.

Methods: Secretome of iRhom2-deficient microglia was analysed by mass spectrometry-based proteomics. Substrates were validated using in vitro assays and primary cells. Phagocytotic activity was measured using immunofluorescence microscopy.

Results: Membrane proteins with reduced ectodomain levels were identified in cell culture supernatants from iRhom2-deficient microglia. Among them, we have validated Trem2, which is involved in regulation of lipid metabolism and phagocytosis. In fact, iRhom2-deficient microglia showed increased phagocytosis and had elevated levels of lipid droplets, suggesting Trem2 proteolysis being iRhom2-dependent and thereby controlling its physiological function.

Conclusions: This study identifies iRhom2/ADAM17 substrates in microglia, pointing to a key role of iRhom2 in regulating key functions such as lipid metabolism and phagocytosis. This highlights proteolysis being a critical process of microglial homeostasis.
SULFORAPHANE NEGATIVELY REGULATES BACE1 EXPRESSION AND RESCUES COGNITIVE IMPAIRMENTS IN AD MOUSE MODELS THROUGH THE ACTIVATION OF NRF2.

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Aims: Finding a new therapeutic target for treatment of Alzheimer's disease (AD)

Methods: Sulforaphane is a phytochemical derived from cruciferous vegetables and it is known as an activator of nuclear factor erythroid-derived 2-related factor 2 (NRF2/NFE2L2). In this study, we administered sulforaphane in AD mouse models, and investigated its therapeutic effects.

Results: Treatment of sulforaphane, an NRF2 activator, could ameliorate cognitive impairment in AD mouse models by decreasing BACE1 expression and Aβ production. This is because NRF2 down-regulates the expression of BACE1 through binding to antioxidant response elements (ARE) in BACE1 promoter.

Conclusions: NRF2 is a negative regulator of BACE1 expression and activation of NRF2 can prevent AD pathogenesis.
Aims: The Alzheimer's disease has been a heavy issue in world health care owing to its difficulty to cure. Despite decades of study, very effective treatment has not been discovered owing to its pathological variation. One of well known and critical pathway is excess of amyloid beta burden in the brain. The amyloid beta is produced from cleavage of amyloid precursor protein (APP) by BACE1, beta secretase. Hence, many researchers have been trying to target BACE1 to find treatment of the disease. The goal of this study was trying to discover novel candidate drugs which may be effective to the disease by screening FDA approved drugs list.

Methods: We screened various FDA approved drugs and chose one chemicals, Chlorhexidine, which showed significant effect on BACE1 expression. To validate the effect of it, we treated it on the Sy5y cells and primary neurons and investigated the expression of BACE1 and its activity via qPCR and western blot. Whether it is working on systemically, we administrated the drugs to AD mouse model, and investigated the cognitive behaviour and amyloid burden.

Results: In vitro experiment, we found significantly decreased BACE1 expression after treating Chlorhexidine, and also decreased cleavage of beta site of APP. This finding is consistent in both Sy5y cells and primary neurons. The effect is also consistent in in vivo experiment. Treatment of the drug improved cognitive function and decreased amyloid burden in AD mice.

Conclusions: All together our research suggested that Chlorhexidine is one of candidate drugs targeting BACE1 for Alzheimer's disease.
BACE INHIBITION AND SYNAPTIC DYSFUNCTION: MECHANISTIC INSIGHTS FROM HUMAN IPSC-DERIVED NEURONS

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Aims: Amyloid precursor protein (APP) β-cleavage by β-site APP-cleaving enzyme (BACE) is believed to be the rate-limiting step of amyloid generation, and therefore a focal target for Alzheimer’s disease (AD) treatment. Several BACE inhibitors succeeded in reducing amyloid load in the brain in clinical trials, but had to be discontinued due to severe side effects, including cognitive worsening and brain atrophy. It has been later shown that BACE inhibition impairs synaptic plasticity and cognitive functions, but the mechanisms are still unknown. Here we test the hypothesis that reduced β-site cleavage of APP in neurons results in accumulation of APP in synapses, leading to impaired neuronal activity.

Methods: We treated human induced pluripotent stem cells (iPSCs)-derived cortical neurons with high-dose BACE inhibitor LY2886721 (Eli Lilly). Secretion of soluble APP fragments in the cell supernatant was measured using sandwich ELISA. For synaptic activity, field action potentials were recorded using a multielectrode array (MEA) system. Protein accumulation was assessed using western blot of cell lysate or synaptosomes or immunocytochemistry.

Results: Treated neurons secreted up to 75% less Aβ40/Aβ42 compared to vehicle control. Soluble APP-β (sAPPβ) was also decreased up to 85%, whereas sAPPα secretion remained unchanged even after 3 days of treatment. BACE inhibition altered neuronal activity and caused progressive increase of intracellular APP levels, only partly in the synaptic terminals.

Conclusions: Accumulation of APP in neurons as a result of missed β-cleavage could explain synaptic dysfunction phenotype caused by pharmacological inhibition of BACE. Unraveling this mechanism is important for a reassessment of such promising treatment strategy.
PRECLINICAL STUDIES ON PHARMACOKINETIC AND PHARMACODYNAMIC PROPERTIES: NEUROPROTECTIVE ACTIVITY OF PHLORETIN FORMULATION IN AB1-42 PEPTIDE-INDUCED ALZHEIMER'S DISEASE.

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Aims: Phloretin, a dihydrochalcone, is a flavonoid displaying neuroprotective, neurotrophic, antioxidant and anti-inflammatory properties when administered as an active pharmaceutical ingredient (API). Alzheimer's disease (AD) is a progressive neurodegenerative disorder for which no definitive cure exists till date. Striatal enriched protein tyrosine phosphatase 61 (STEP61), a brain-specific protein phosphatase, is a significant regulator of synaptic protein phosphorylation/dephosphorylation. When explored for docking studies in silico, phloretin is demonstrating good docking score and interaction with STEP61. In this regard, our aim is to correlate the pharmacokinetics (PK) and pharmacodynamics (PD) data of prepared formulation, assessing its therapeutic effect on the AD biomarkers.

Methods: Docking studies were performed using Schrödinger Drug Discovery Suite 2019. Docking score was used for binding affinity prediction and phytoconstituents ranking. The formulation of phloretin is in the developmental phase. For preclinical PK/PD correlation of phloretin formulation, rat model will be used. The PK parameters of phloretin formulation will be evaluated. Immunohistochemistry of STEP61, NMDARs and AMPARs (subunits of glutamatergic receptors) and ERK1/2, p38 (subunits of Mitogen-Activated Protein Kinases [MAPK]), Brain Derived Neurotrophic Factor (BDNF) and synaptophysin will be performed to examine these markers in the hippocampus. In addition, different inflammatory mediators and antioxidant markers will be analyzed and compare with donepezil as a standard drug.

Results: Docking score if Phloretin was found to be -5.115. Development of phloretin formulation is on-going.

Conclusions: The study implicates that phloretin formulation could be used as a promising disease-modifying strategy in therapeutic use for AD.
POSTERS

TRANSFERRIN FUNCTIONALIZED NANOSTRUCTURED LIPID CARRIERS FOR BRAIN TARGETING OF RIVASTIGMINE AND RESVERATROL FOR ALZHEIMER’S DISEASE THERAPY

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Aims: The multiple underlying causes of Alzheimer’s disease (AD) necessitates combination drug therapy, targeting multiple mechanisms of disease pathogenesis. Here, we have employed an acetylcholinesterase inhibitor Rivastigmine hydrogen tartrate (RHT) and a polyphenol Resveratrol (RSV) proven to have beneficial effects in AD. The objective is to formulate and characterize Transferrin (Tf) functionalized nanostructured lipid carriers (Tf-NLCs) of RHT and RSV for brain targeting.

Methods: The NLCs were synthesized by w/o/w double emulsion method and optimized using Central Composite Design. The conjugation of NLCs with Tf was achieved by co-incubating in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide. The prepared NLCs and Tf-NLCs were characterized for particle size, polydispersity index (PDI), entrapment efficiency (EE), drug release, transmission electron microscopy (TEM), Fourier Transform Infrared Spectroscopy (FTIR). Tf conjugation efficiency was determined by Bradford assay.

Results: Optimized NLCs had a particle size of 70.8±10.7 nm, PDI of 0.186±0.088 and drug EE of 99.1±0.4% for RSV and 40.3±5.2% for RHT. NLCs exhibited sustained release of drugs in vitro for 48h. After Tf conjugation, the particle size increased, EE demonstrated no significant change and Tf-NLCs depicted more sustained release than NLCs. TEM ascertained spherical morphology, FTIR and Bradford assay proved the functionalization of NLCs with Tf.

Conclusions: Dual drugs loaded Tf-NLCs may evolve as a superior treatment outcome for AD. The in vitro findings are encouraging and advocate the potential of Tf-NLCs in amplifying receptor mediated drug delivery that would be further substantiated by the in vivo studies.
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Aims: Alzheimer’s disease (AD) is defined by the accumulation of Aβ plaques and tau tangles. Impaired synaptic function and disrupted endolysosomal vesicular trafficking precede Aβ and tau pathology in AD patients. The greatest risk factor for late onset AD is ApoE4, yet an underlying molecular mechanism is still unclear. ApoE4 impairs synaptic plasticity, implicating a potential mechanism of accelerated AD onset in ApoE4 carriers. The first aim of this study is to identify the underlying cause that enhances AD risk in ApoE4 carriers. The second aim of this study is to establish a potential therapeutic target to restore the ApoE4-risk. More specifically, how the inhibition and depletion of Sodium/Hydrogen exchanger (NHE) 6 – a proton leakage channel that resides and alkalizes the early endosomes – affects ApoE4 kinetics. Lastly, our third aim of this study is to determine how NHE6 depletion affects Aβ plaque accumulation.

Methods: We utilized primary neuronal cultures (Chen et al., 2005), surface biotinylation experiments (Chen et al., 2010; Xian et al., 2018), production of NHE6-KO mouse model (Pohlkamp et al., 2021), and immunohistochemistry to evaluate whether NHE6 depletion restores ApoE4-dependent synaptic dysfunction and Ab plaque deposition.

Results: We identified impaired vesicular trafficking as a key mechanism by which ApoE4-mediated synaptic dysfunction manifests itself. In addition, the ApoE4-mediated synaptic dysfunction is rescued by inhibition and depletion of NHE6 (Xian et al., 2018). Lastly, NHE6 ablation substantially reduces Aβ plaque accumulation in the APP-NL/F mouse line.

Conclusions: We have identified and validated a novel therapeutic target for preventing or delaying Alzheimer’s disease in ApoE4 carriers.
ETHANOLIC EXTRACT OF ZANTHOXYLUM SP. REDUCED TAU BURDEN, NEUROINFLAMMATION, AND INCREASED APOE EXPRESSION IN THE TRIPLE TRANSGENIC MICE MODEL OF ALZHEIMER’S DISEASE

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Aims: To evaluate the potential beneficial effect of an ethanolic extract from Zanthoxylum sp. in the 3xTg-AD mouse model of AD.

Methods: Twelve months old 3xTg-AD mice were treated for 18 weeks with ethanolic extract of Zanthoxylum sp., (25 mg/kg/day). Immunohistochemical changes (AT8, Iba-1, ApoE staining) were analyzed by confocal microscopy. Results were compared with control animals and 3xTg-AD mice treated with vehicle (water).

Results: Treated animals showed a reduction in immunoreactivity AT8 antibody, as well as decreased neuroinflammation across all hippocampal regions. These changes were accompanied by increased expression of ApoE, as compared to untreated animals.

Conclusions: Zanthoxylum sp. ethanolic extract reverses cognitive impairment in 3xTg-AD mice associated with reduction in neuroinflammation, pathological Tau burden and increased ApoE expression.
Aims: Prolactin-releasing peptide (PrRP) is a neuropeptide with anorexigenic and antidiabetic properties. Because of the suggested link between obesity and/or type 2 diabetes and Alzheimer’s disease (AD) development, anorexigenic and/or antidiabetic compounds were repurposed as potential neuroprotective compounds.

Methods: In this study, we focused on exploring and further clarifying the mechanisms of interconnection between obesity and associated metabolic disturbances, in particular obesity, insulin resistance and T2DM and age-related neurodegeneration in the APP/PS1 mouse model of AD fed with high fat (HF) diet. Furthermore, the potential neuroprotective properties through activation of anti-inflammatory pathways of PrRP31 and palm11-PrRP31 were studied using this model.

Results: We observed worsened Aβ pathology and increased neuroinflammation in APP/PS1 mouse model fed with HF diet than in APP/PS1 mouse model fed with standard chow diet. Furthermore HF diet increased peripheral inflammation, measured by c-reactive protein and insulin resistance, measured with oral glucose tolerance test, a major possible players in the development of brain insulin resistance and neurodegeneration. Lipidized analog of PrRP ameliorated obesity and T2DM by improving brain insulin resistance and modulated the energy balance signaling. Furthermore, PrRP analogs improved neurodegenerative and neuroinflammatory changes in the brain.

Conclusions: In summary, lipidized analogs of PrRP seem to be potential neuroprotective agents but the exact mechanism of action must be further studied.
NOVEL SOLUBLE TNF INHIBITOR IMPROVES OUTCOMES IN A MOUSE MODEL OF TRAUMATIC BRAIN INJURY-INDUCED ALZHEIMER DISEASE

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Aims: Traumatic brain injury (TBI) is a known risk factor for the later development of Alzheimer’s disease (AD). Unfortunately, there are no therapies to ‘cure’ TBI or AD, and any drugs to improve symptoms have numerous side-effects. Therefore, new pharmacotherapies without side-effects are urgently needed. A major inflammatory cytokine upregulated following TBI and involved in AD pathology is Tumor Necrosis Factor (TNF). The transmembrane form of TNF (tmTNF) preferentially binds TNFR2 promoting predominantly beneficial outcomes (blocking its activity may cause immunological and cardiac dysfunction), while the soluble form of TNF (solTNF) preferentially binds TNFR1 promoting detrimental brain outcomes, including neuronal cell death, amyloid beta plaque and tau neurofibrillary tangle pathology. While traditional TNF inhibitors are non-selective at blocking TNFR1 and TNFR2 activity, a novel second-generation TNF inhibitor (XPro1595, INmuneBio Inc) selectively inhibits only solTNF/TNFR1 activity. In a clinical trial in cancer patients XPro1595 was safe and well-tolerated, and in a second clinical trial in AD patient’s interim data shows XPro1595 reverses brain WM neuroinflammatory levels. While TBI accelerates the onset of AD, the role of solTNF/TNFR1 activity in TBI-induced AD pathology has remained unknown.

Methods: AD transgenic mice (3xTg-AD) underwent TBI (CCI injury model), treated with XPro1595 (10 mg/kg, S.C.) 30 minutes post-injury, and allowed survive for 24 hours.

Results: Preliminary data suggests XPro1595 treatment prevents injury-induced increases in glial reactivity (GFAP), APP and amyloid beta (6E10), Tau (Tau46), and phospho-Tau (AT8 and PHF-1) expression.

Conclusions: This data supports the use of XPro1595 clinically following TBI to prevent the later development of AD pathology.
SYNTHESIS AND EVALUATION OF NOVEL SERIES OF VINYL SULFONE DERIVATIVES FROM VEDA-1209 AS PROMISING NRF2 ACTIVATORS FOR ALLEVIATING NEUROINFLAMMATION

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Aims: Accumulated oxidative damage is related to neurodegenerative condition such as Alzheimer’s disease (AD) and Parkinson’s disease (PD). Indeed, decrease of expression of the nuclear factor E2-related factor 2 (Nrf2) have been reported in AD brain. Nrf2 plays a vital role in anti-inflammatory responses as well as antioxidant defenses by mediating the expression of various antioxidant enzyme genes and pro-inflammatory cytokines. So Nrf2 activation has emerged as a therapeutic target for neurodegenerative diseases. In this work, a series of novel Veda-1209 derivatives were synthesized by substituting vinyl sulfone and screened for their Nrf2 activating efficacy.

Methods: Synthesized vinyl sulfone compounds were assessed for their Nrf2 activating efficacy, antioxidant and anti-inflammatory effects in vitro. A optimal compound was used for in vivo study of memory impairment recovery in a scopolamine-induced mouse model.

Results: Among the synthesized compounds, a potent compound (6e) showed superior Nrf2 activation compared to Veda-1209 (Nrf2 activation EC⁵₀: Veda-1209 = 625 nM vs compound 6e = 38 nM). Unlike insufficient drug-like properties of Veda-1209, 6e exhibited better drug-like properties including stability and CYP enzyme interaction. Furthermore, memory impairment was recovered with administration of 6e in a scopolamine-induced mouse model.

Conclusions: 6e, a new vinyl sulfone compound synthesized based on the structure of Veda-1209, was confirmed to have proper drug-like properties, including an potent Nrf2 activation effect.
POSTERS

PHARMACOLOGICAL INHIBITION OF SOLUBLE EPOXIDE HYDROLASE MODIFIED TRANSCRIPTOME PROMOTING MEMORY REINSTATEMENT THROUGH REDUCTION OF AD-HALLMARKS IN 5XFAD.

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Aims: It has been demonstrated the upregulation of soluble epoxide hydrolase (sEH) in Alzheimer’s disease (AD) brains as well as its inhibition as therapeutic strategy for AD. In this study, we tested a potent and selective sEH inhibitor (UB-SCG-51) to show its neuroprotective effects, deciphering new molecular pathways in 5xFAD mice and in vitro model.

Methods: Wild-type and 5xFAD (n=36) male mice 28-weeks-old were used. UB-SCG-51 was dissolved in 1.8% 2-hydroxypropyl-β-cyclodextrin and administered by gavage (5 mg/kg/day). After 4 weeks of treatment, behavioural tests were performed to evaluate the cognitive state as well as molecular analysis of AD hallmarks through WB, qPCR and IHC. In addition, a transcriptome analysis using RNA- and miRNA-seq was executed to evaluate changes in DNA transcription. To determine if sEH inhibitor prevents neurotoxic reactive-astrocyte conversion, primary mouse astrocytes were pre-treated in vitro with UB-SCG-51 followed by a combination of TNFα, II-1β and C1q (T/I/C) for 24 hours.

Results: Behavioural tests showed that UB-SCG-51 rescued 5xFAD cognitive impairment and it significantly reduced neuroinflammation and apoptotic markers. Likewise, a reduction in the number of Aβ plaques and Tau hyperphosphorylation was found in 5xFAD treated mice. Interestingly, transcriptomic changes also were detected in treated group. In vitro, UB-SCG-51 prevented the expression of genes associated with reactive astrocytes and inflammation induced by T/I/C.

Conclusions: Our results demonstrated that sEH inhibition by UB-SCG-51 promoted transcriptome modulation, leading to cognitive improvement as well as a reduction in neuroinflammation and AD-hallmarks in 5xFAD mice and in vitro astrocytes models.
Aims: Sporadic Alzheimer’s disease (SAD) is the most commonly prevalent dementia and progressive neurodegenerative disease. A polyphenolic flavonoid, 7,8-dihydroxyflavone (7,8-DHF) was used to reverse the cognitive deficit in a rat SAD model by reversing oxidative imbalance, mitochondrial enzyme dysfunction, and insulin resistance.

Methods: For the SAD model, streptozotocin (STZ-3 mg/kg) was injected intracerebroventricularly (ICV) in male Wistar rats to induce cognitive dysfunction. Cognitive functions were evaluated by Morris water maze (MWM) and novel object recognition (NOR) tests, while locomotor activity was determined in actophotometer. 7, 8-DHF was given orally in doses of 5 mg/kg, 10 mg/kg, and 20 mg/kg, and reference standard drug rivastigmine in a dose of 2 mg/kg. Antioxidant enzymes, mitochondrial enzyme complexes were determined biochemically, insulin-degrading enzyme (IDE) and p-tau by ELISA, and histopathology by H&E staining.

Results: 7,8-DHF attenuated cognitive deficit induced by ICV-STZ in MWM and NOR. Moreover, in the cortex and hippocampus regions of the brain, levels of reduced glutathione, catalase, superoxide dismutase, and mitochondrial complex enzymes and increased lipid peroxidation, protein carbonylation, and nitrite levels were subsequently reversed by 7,8-DHF and rivastigmine. IDE and p-tau protein were found to be altered. Histopathological examination revealed halted neurodegeneration.

Conclusions: Conclusively, 7,8-DHF was found to be neuroprotective in the ICV-STZ rat model of SAD by ameliorating oxidative stress, mitochondrial dysfunction, and insulin resistance, thereby improving cognitive functions evident with the behavioral results. Hence, these results can further have clinical relevance in human AD.
Aims: Impaired cholinergic neurotransmission and increased cerebral beta amyloid depositions and oxidative stress play a crucial role in the pathogenesis of dementia. Therefore, exploring drugs that could target these pathological markers seems to be an efficient way of managing dementia. Quercetin 4'-O-glucoside (Q4G) is a natural occurring flavonoid which is reported to have strong antioxidant mediated neuroprotective effects. However, its possible beneficial effects in improving cognitive functions are yet to be determined. Therefore, the present study was designed to evaluate the memory improvement effects of Q4G in streptozotocin induced dementia in mice.

Methods: Q4G (10 and 20 mg/kg, p.o., once daily) was administered for 2 weeks following icv-STZ injection. Morris water maze was used to access the memory functions of animals while brain biochemical parameters including brain beta amyloid levels, acetylcholinesterase activity, lipid peroxidation and glutathione levels were determined to address the neuroprotective mechanism of action.

Results: Treatment with Q4G showed improved memory functions of animals via reducing beta amyloid, acetylcholinesterase activity and oxidative stress in mice brain.

Conclusions: Q4G could be developed as a neuroprotective drug for the management of dementia after further thorough investigations.
Ψ-Glutamyl-transpeptidase resistant glutathione analog attenuates progression of Alzheimer’s disease-like pathology and neurodegeneration in a mouse model

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Aims: Oxidative stress in Alzheimer’s disease (AD) is mediated, in part, by the loss of Glutathione (GSH). Previous studies show that γ-glutamyl transpeptidase (GGT)-resistant GSH analog, Ψ-GSH, improves brain GSH levels, reduces oxidative stress markers in brains of APP/PS1 mouse model of AD. Herein, we examined whether Ψ-GSH can attenuate the disease progression when administered following the onset of AD-like pathology in vivo.

Methods: Cohorts of APP/PS1 mice were administered a Ψ-GSH for 2 months starting at 8 month or 12 months of age. Post-mortem immunohistochemistry and stereological analyses of key AD markers were performed. Levels of oxidative stress were measured in tandem.

Results: We show that Ψ-GSH treatment was able to dramatically reverse indices of oxidative stress in older mice by restoration of enzyme Glyoxalase-1 (Glo-1) activity and reduces levels of insoluble Aβ. Quantitative neuropathological analyses show that Ψ-GSH treatment significantly reduces Aβ deposition and brain inflammation in APP/PS1 mice. More importantly, Ψ-GSH treatment attenuated the progressive loss of cortical TH+ afferents and the loss of TH+ neurons in the locus coeruleus (LC).

Conclusions: Collectively, chronic Ψ-GSH treatment, even when applied when AD-like pathology is established in aged APP/PS1 mice, can reverse oxidative stress and attenuate progressive AD pathology, including inflammation and neurodegeneration in vivo. Ψ-GSH efficiently engaged with the target Glo-1 enzyme and counteracted glycation-induced oxidative and inflammatory pathology even in animals with ongoing pathology and neurodegeneration. Thus, Ψ-GSH represents a viable strategy for development of future AD therapeutics and supports further investigations for advancement of ψ-GSH to tackle this devastating disease.
EFFECTS OF NXP031 THAT INCREASED THE EFFICACY OF VITAMIN C IN THE 5XFAD MOUSE MODEL

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Aims: The purpose of this study is to investigate the effect of amyloid-β (Aβ) accumulation and memory impairment after administering NXP031 (Vitamin C-Aptamer Complex) to the 5XFAD model.

Methods: The aptamer is a single-stranded DNA that can selectively bind to a target molecule and is isolated by the SELEX method and was provided by Nexmos Co., Ltd. L-Ascorbic acid (Sigma-Aldrich, MO, USA) was added at a ratio of 1:50 (w/w) and then adjusted to pH 5.7 to form the final NXP031 (ascorbic acid: aptamin = 200mg/4mg/kg). Groups were assigned a 10-month-old wild type (WT), 5XFAD (Tg), Tg + Vitamin C, and Tg + NXP031. Vitamin C and NXP031 were administered intraperitoneally for 8 weeks every other day. After the cognitive-behavioral experiment using the Y-maze test, mice were sacrificed and evaluated for Aβ plaques, cholinergic neurons (ChAT), Iba1 (microglia), and neprilysin by hippocampal immunohistochemistry.

Results: In the Tg + NXP031 group, improved spatial working memory, and the accumulation of Aβ was decreased in the cortex, hippocampal CA1, and dentate gyrus region compared to the Tg group. Cholinergic neurons were rescued, microglial activation was significantly reduced in the Tg + NXP031 group. Also, neprilysin was increased in the NXP031 group, and ROS was decreased.

Conclusions: Since NXP031 compensates for the weakness of vitamin C, high antioxidant efficacy can be expected. The results of this study suggest that NXP031 has therapeutic potential contributing to the recovery of memory deficits caused by Alzheimer's disease.
**EFFECT OF AMYLOID-B (Aβ) PLAQUE CLEARANCE OF NXP031 IN THE 5XFAD MOUSE MODEL**

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**Aims:** The purpose of this study is to investigate the effect of amyloid-β (Aβ) plaque degradation and memory impairment after administering NXP031 (Vitamin C-Aptamer Complex) to the 5XFAD model.

**Methods:** The aptamer is a single-stranded DNA that can selectively bind to a target molecule and is isolated by the SELEX method and L-Ascorbic acid was added at a ratio of 1:50 (w/w) and then adjusted to pH 5.7 to form the final NXP031 (ascorbic acid: aptamin = 200mg/4mg/kg). Groups were assigned a 10-month-old wild type (WT), 5XFAD (Tg), Tg + Vitamin C, and Tg + NXP031. Vitamin C and NXP031 were administered intraperitoneally for 8 weeks every other day. After the cognitive-behavioral experiment using the Y-maze test, mice were sacrificed and evaluated for Aβ plaques accumulation, cholinergic neurons (ChAT), Iba1 (microglia), Nrf-2, GSTO1/2, and proteases (neprilysin, MMP-2, MMP-9) in the hippocampus and cerebral cortex.

**Results:** In the Tg + NXP031 group, improved spatial working memory in the Y-maze test, and the accumulation of Aβ was decreased in the cortex, hippocampal CA1, and dentate gyrus region compared to the Tg group. The activity of choline acetyltransferase in the Medial Septum were rescued, microglial activation was significantly reduced in the Tg + NXP031 group. Also, Nrf-2, GSTO1/2, neprilysin, MMP-2, MMP-9 were significantly increased in the Tg + NXP031 group, and ROS was decreased compared to the Tg group.

**Conclusions:** After 8 weeks of treatment with NXP031, Aβ plaques were significantly reduced, antioxidant effects, and protease activation, suggesting a strong potential as a therapeutic for Alzheimer's disease.
FERROPTOSIS, A RECENTLY IDENTIFIED CELL DEATH, AS A THERAPEUTIC TARGET FOR PARKINSON'S DISEASE.

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Aims: Parkinson’s disease (PD) is characterized by a progressive degeneration of dopaminergic neurons (DopN) in the SN associated with an iron overload and lipid peroxidation (LPO). Recently, a novel form of regulated cell death (RCD), named ferroptosis, has been described as an iron-dependent RDC caused by an accumulation of lipid peroxides to lethal levels in the cellular membranes. During ferroptosis, LPO occurs in polyunsaturated fatty acids (PUFAs) of cell membranes, in particular Arachidonic Acid (AA). We aim to determine the pathophysiological conditions that induce ferroptosis and neuroprotective targets in our cell model

Methods: LUHMES cells a human DopN model, were treated with AA, Fe, AA+Fe or RSL3 (ferroptosis inducer). At 24h, we measured LPO by flow cytometry and at 48h we measured cell viability by resazurin assay or flow cytometry. We used the same protocol after pretreating with antiferroptotic drugs or silencing ACSL4, 15/15B lipoxygenases (15/15B-LOX) with siRNA, three proferroptotic genes

Results: A supplementation of AA or Fe alone was not toxic, but AA+Fe treatment induced high mortality and LPO levels. Pharmacological or genetical inhibition of the ferroptosis rescued cells from AA+Fe toxicity, to same extent as they rescue against RSL3. These results suggest that LUHMES are susceptible to ferroptosis induced by a supplementation of low levels of AA and Fe.

Conclusions: Several regulatory mechanisms have been described in ferroptosis including iron and lipid metabolisms. Modulating the lipid composition of membranes associated with an iron overload induces specifically ferroptosis in Luhmes. These results could lead to neuroprotective strategies in PD by blocking ACSL4 and 15/15B-LOX
RESISTANCE EXERCISE REDUCES THE HYPERLOCOMOTION, CORTICOSTERONE LEVELS, AND AMYLOIDE PLAQUES IN THE HIPPOCAMPUS OF APP/PS1 TRANSGENIC MICE

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Aims: Objective: Physical exercise has neuroprotective effects stimulating neurogenesis, neurotrophic factors, and neuroplasticity, and modulating inflammatory responses in Alzheimer’s disease (AD). Most studies, however, are performed with aerobic exercises, and few have investigated the effects of other modalities that also show positive effects on AD, such as resistance exercise (RE). Similar to AD patients, the double-transgenic APPswe/PS1dE9 (APP/PS1) mice exhibit amyloid-β (Aβ) plaques in the cortex and hippocampus, hyperlocomotion, cognitive deficits, and exacerbated inflammatory response. Therefore, the present study aimed to investigate the effectiveness of RE training in the prevention and recovery of neuropathological conditions of APP/PS1 mice.

Methods: Adult APP/PS1 and wild type (WT) mice were divided into three groups: WT, APP/PS1, APP/PS1+RE (n = 15/group). RE training lasted four weeks and, at the end of the program, animals were tested in the open field test for locomotor activity and in the object recognition test for recognition memory evaluation. The brains were removed for immunohistochemical analysis of Aβ plaques, and blood was collected for plasma corticosterone by ELISA assay.

Results: Statistical analysis indicated that RE program reversed the hyperlocomotor behavior, but not the recognition memory, reduced the corticosterone levels (p<0.05) and the number of Aβ plaques in the hippocampus (p<0.05) of APP/PS1 mice (p<0.05).

Conclusions: Conclusion: Altogether, this study demonstrated the beneficial effects of resistance exercise training as a complementary treatment of AD.
Aims: An observational study in clinical practice about multisystem neurodegenerative disease, with a predilection in the intellectual population, and it is controllable and curable to a fair level with pharmaceutical agents.

Methods: The study population is small with 9 patients over a period of 2020-21. Which was observed with an assessment with clinical improvement, imaging, and biochemical parameters, including vitamin assay, and in 2% of cases CSF study. All these cases have been treated pharmaceutically with therapeutic doses of Edravon, Vitamin B12 via Nasal spray, and Cerebroprotein hydrolysate, for a period of 36 days from the date of diagnosis. Repeat imaging study will be taken after a period of 9 months.

Results: It is observed that within a period of 90 days, there is a significant improvement in the clinical parameters and ADL, and in some percentage, there is total arrest of the progress of the symptoms.

Conclusions: Neurodegenerative disease is an uncommon disease, and its identification in intellectuals over a percentage of 80%, in the observed cases, and responding to the pharmaceutical agents, to a fair extent, denote the etiological factors may be multifactorial like vitamin B12 deficiency, mitochondrial dysfunction, and reduction in the intranuclear nucleoprotein.
E2511, A NOVEL SMALL COMPOUND TRKA BIASED POSITIVE ALLOSTERIC MODULATOR, REINNERVATES CHOLINERGIC NEURON VIA ENHANCEMENT OF SPECIFIC TROPHIC SIGNALING OF TRKA IN NON-CLINICAL STUDIES

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Aims: Cholinergic innervation is particularly vulnerable in many neurodegenerative diseases associated with cognitive dysfunction, and loss of cholinergic neurons in Alzheimer’s disease (AD) is an early pathogenic event correlated with cognitive impairment. Nerve growth factor (NGF) plays a major role in the maintenance and function of cholinergic neurons. The activation of NGF-induced trophic signaling could be a promising therapy for AD, reinnervating and maintaining cholinergic neurons. We provide new evidence that E2511, a novel small compound TrkA biased positive allosteric modulator, induces functional restoration of cholinergic neuronal network.

Methods: E2511 was characterized by biochemical studies evaluating the phosphorylation level of TrkA using primary neuron cultures. The trophic effect of E2511 on cholinergic neurons was assessed by biochemical studies and immunohistochemistry using cholinergic markers in normal rats and human Tau P301S transgenic mice (Tau tg mice).

Results: E2511 enhanced genes expression level relating to cholinergic functions such as choline acetyltransferase via increase in phosphorylation level of TrkA in vivo, but no increase in pain-related genes was detected. In addition, the effect of E2511 on genes expression level was potentiated by repeated dosing. Furthermore, chronic administration of E2511 at doses showing increase in genes expression restored damage of cholinergic neuron in the septum of Tau tg mice, and cholinergic presynapse in hippocampus was reinnervated by chronic administration of E2511 at the dose.

Conclusions: E2511 enhances cholinergic function by increasing TrkA signaling as biased positive allosteric modulator, and E2511 functionally reinnervates cholinergic neuronal network in Tau tg mice.
CHARACTERIZATION OF HIPPOCAMPAL BDNF ENCAPSULATED CELL BIODELIVERY IN THE NOVEL APP NL-G-F MOUSE MODEL OF ALZHEIMER'S DISEASE

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Aims: Alzheimer’s disease (AD) is an age-related disease characterized by synaptic dysfunction, brain inflammation, and neurodegeneration against which there is no effective cure available. Brain-Derived Neurotrophic Factor (BDNF) is a key molecule involved in the learning and memory process, with a crucial role in synaptic plasticity and neuronal survival. Several findings support that a reduced BDNF expression is associated with AD pathogenesis. BDNF has been proposed as a potential therapy for AD, but BDNF has a low brain permeability. In this study, we used an innovative encapsulated cell biodelivery (ECB) device capable to locally deliver BDNF to characterize its neuroprotective effects in the novel AD APP-knock-in mouse model AppNL-G-F.

Methods: ECB devices were surgically implanted in the hippocampus of 3-month-old AppNL-G-F mice. BDNF effects on AD pathology were evaluated after 1, 2, and 3 months of treatment by immunohistochemical and biochemical analysis. In the 3 months-treated groups, cognitive and memory performances were also evaluated.

Results: The ECB implants were well tolerated with no signs of unwanted side effects or weight loss. A high BDNF staining was detected in most of the brains in the area surrounding the devices at 1 and 2 months but retrieved devices showed variability of secretion. Furthermore, 3 months of BDNF treatment significantly improved AppNL-G-F mice’s spontaneous behavioral alternations in the Y-maze test.

Conclusions: The results of this study are encouraging and support the BDNF device as a promising approach for treating cognitive AD decline. Optimization of the mouse-sized devices to reduce variability of BDNF release is needed for future experiments and clinical translation.
ASSESSMENT OF ADAPTIVE IMMUNE RESPONSE AGAINST ENCAPSULATED CELL BIODELIVERY DEVICES RELEASING NEUROTROPHIC FACTORS

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Aims: Drug delivery to brain tissue had been a major challenge for regenerative therapies. This is due to large size of neurotrophic molecules, unable to cross the blood-brain barrier when injected peripherally. Furthermore, long-term in-situ delivery within brain tissue using cell mediated (stem cells or encapsulated) drug delivery strategies encountered major setback due to immune reaction from the host. We previously utilized the encapsulated cell biodelivery (ECB) platform to deliver mature nerve growth factor in Alzheimer’s disease patients and observed altered release with time. In this study, we evaluated the immunogenic potential of ECB devices releasing human brain derived neurotrophic factor (BDNF).

Methods: Since immunogenic reactivity is increased in cross species interaction, we used mouse ex-vivo splenocytes culture, incubated with empty device (controls) and devices containing engineered cell line (ARPE-19) for 48hr. We then examined activation and intracellular cytokines level in immune cells from adaptive immunity (T and B cells) by flowcytometry.

Results: Immune cells cultured with ECB devices (controls or cell encapsulated) expressed similar level of CD69 expression as unstimulated immune cells and did not produce cytokines including TNF-a and IFN-g. However, PMA-ionomycin treatment ensured that immune cells could mount an immunogenic response.

Conclusions: The interaction of mouse immune cells to antigens shed by the human cells encapsulated within ECB device did not lead to their activation nor altered functional status in an ex-vivo set-up. However, further work is warranted to study in-vivo immunogenicity of ECB devices in brain tissue.
ENCAPSULATED CELL BIODELIVERY OF NEUROTROPHIC MOLECULES - OPTIMIZATION OF CELL BEHAVIOR


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Aims: Drug delivery to brain tissue is a challenge, especially for neurotrophic molecules which does not cross the blood-brain barrier (BBB). We developed and utilized an encapsulated cell biodelivery (ECB) platform to deliver various neurotrophic molecules - NGF, BDNF and GDNF, in pre-clinical studies. In a phase 1b clinical trial in Alzheimer's disease (AD) patients, ECB mediated human mature NGF (hmNGF) delivery alleviated various AD symptoms, but hmNGF release were found altered over-time. In this study, we evaluate some of the reasons which may affect ECB function.

Methods: Human retinal epithelial cells (ARPE-19) were genetically modified to release hmNGF (termed NGC0211) and cultured without splitting (20-30 days), to mimic the inherent space constraint within ECB devices. Various parameters were studied including prolonged cell proliferation, contact inhibition, cell death, re-population, and debris clearance using microscopy. For debris clearance, dying cells (CFSE labelled) was plated on healthy cells (CellMask™ Deep Red plasma membrane stain). NGC0211 cells were exposed to inflammatory cytokines and cell proliferation was checked by ki67 staining.

Results: NGC0211 cells divides until contact inhibition is achieved, but growth pattern differed for different culture medium. Under serum-free stress conditions, some cells undergo death (around day14) but newer cells proliferate to fill-up the space. Inflammatory molecules (TNFα and IFNg) hampered cell proliferation severely.

Conclusions: NGC0211 cells undergo phasic death and repopulation under in-vitro serum-free stress conditions and repopulation by proliferation is hampered by inflammatory molecules. Internal and external factors may therefore dictate the overall ECB mediated neurotrophin delivery in-vivo.
BRAIN TPS STIMULATION IN ALZHEIMER DISEASE AND OTHER NEUROLOGICAL PATHOLOGIES

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Aims: To analyze the evolution of patients with Alzheimer's disease (AD), frontotemporal dementia, Parkinson plus, and in patients with cognitive impairment suffered after TBI, by means of treatment with transcranial shock waves (TPS; Neurolithe, Storz Medical).

Methods: Patients: Three AD (early disease in two cases and very advanced in the other). One patient suffering fronto-temporal dementia. One patient suffering Parkinson plus, and two patients that had significant cognitive impairments after TBI occurred 2 and 8 years before. Several neuropsychological tests were carried out pre-TPS treatment and repeated monthly after every TPS cycle and 6 months later. TPS treatment: 6000 transcranial pulses per session (session: 30 minutes, 3 times/week during 2 weeks). After one month resting a second cycle was performed. Follow up: 6 months later.

Results: A significant cognitive improvement was observed in the two early AD (orientation, independence, verbal fluency), while no changes were observed in the other AD patient, although the negative course of the disease seemed to be interrupted. No cognitive changes were found in the patient with fronto-temporal dementia or in Parkinson plus (but spasticity improved in her). Very significant cognitive changes were found in both TBI patients.

Conclusions: Although this study has many limitations (small number of patients and heterogeneity), our data indicate that TPS is useful for early and mild AD, and cognitive impairments after a TBI. TPS is safe and it can be useful not only for AD but also for problems associated to TBI (including spasticity), although more patients need to be analyzed.
Aims: Epigenetic alterations are a fundamental pathological hallmark of Alzheimer’s disease (AD), where histone modifiers and their marks are dysregulated. Herein, we uncover the unknown G9a modulation pathways involved in AD, demonstrating how H3K9me2 influences the gene expression in the neurodegenerative process.

Methods: Female and male of SAMP8 and SAMR1 24 weeks old were used to perform cognitive and molecular studies. We divided these mice randomly into three groups: SAMR1 (n = 17), SAMP8 Control (n = 17), and SAMP8 treated with UNC0642 (SAMP8 UNC0642 (5 mg/Kg); n = 17). After 2 weeks of treatment, behavioral tests were performed to evaluate the cognitive state as well as molecular analysis of AD hallmarks through WB, qPCR and Golgi Staining. In addition, a transcriptome analysis was executed to evaluate changes in DNA transcription. To determine if G9a inhibitor prevents inflammation, primary mixed culture cells were performed.

Results: We found that G9a and H3K9me2 are upregulated in AD brain patients, correlating with increased Aβ42/Aβ40 ratio. Likewise, treatment with the G9a inhibitor, UNC0642, in SAMP8 mice reversed high levels of H3K9me2, and rescued the cognitive decline. Interestingly, an exploratory H3K9me2 ChIP-seq analysis demonstrated that during treatment, the histone mark H3K9me2 is enriched at promoters of genes associated with neural functions. Furthermore, a transcriptional profile analysis revealed the neuronal plasticity activation and a reduction of oxidative stress and neuroinflammation, the latter being also validated by cell cultures.

Conclusions: Our findings confirm that G9a inhibition promote a positive outcome in AD, being a promising therapeutic strategy.
RESULTS FROM A SINGLE ASCENDING DOSE STUDY IN HEALTHY VOLUNTEERS OF ACD856, A POSITIVE MODULATOR OF NEUROTROPHIN TRK-RECEPTORS

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Aims: ACD856 is a novel positive allosteric modulator of Trk-receptors in clinical development for the treatment of Alzheimer’s disease and other disorders where cognition is impaired. The aim of this study was to assess the safety, tolerability, and pharmacokinetics (PK) of single ascending doses of ACD856.

Methods: 48 healthy volunteer subjects were administered ACD856 or placebo as an oral solution in a fasted state in 6 cohorts of stepwise ascending doses. After each dose, the safety, tolerability, and pharmacokinetics of ACD856 were assessed by an internal Safety Review Committee which decided on the escalation of dose to the next cohort. Additionally, food effect on the PK properties was assessed in 6 subjects participating in a fed cohort.

Results: There were no clinically significant changes from baseline in mean ECG, vital signs, physical examination findings, clinical chemistry, haematology, coagulation, or urinalysis parameters after receiving the single dose of ACD856. Occasional individual abnormal laboratory values were observed but all were assessed as not clinically significant. Most of the reported adverse events were of mild intensity, only a few were of moderate intensity and none of severe intensity. No serious adverse events were reported. The pharmacokinetic data showed a rapid absorption, long half-life, high bioavailability, and a linear dose-dependent exposure.

Conclusions: ACD856 was shown to be safe and well tolerated in man at the tested dose levels and with a suitable pharmacokinetic profile for further clinical development. In the next step, ACD856 is evaluated in a multiple ascending dose study.
Aims: Alzheimer's disease has been associated with latent herpes virus reactivation. Identifying herpes virus-recruited host proteins during its assembly allows the interrogation fundamental cellular events leading to associated "sporadic" Alzheimer's disease.

Methods: In vitro infections with herpes simplex virus 1 of vero and human neuroblastoma cells, including CRISPR/Cas9-based gene knockouts. Recombinant protein expression and NMR structure determination. Heterologous gene expression of mutant tau. iPSC 2D cultures and human brain organoids. Sandwich ELISA.

Results: A host protein-targeted small molecule drug highly active against herpes simplex virus 1 (HSV-1) infection in human brain organoids and cell lines was identified to interact with macrophage migration inhibitory factor (MIF) where it acted by intercalating between MIF units within a trimer, as determined by nuclear magnetic resonance (NMR). From post-mortem brain homogenates of patients with Braak 6-staged AD the small molecule lead compound specifically eluted a MIF subpopulation that correlated with the oxidized conformer of MIF (oxMIF). HSV-1 led to an increase in tau phosphorylation at distinct residues, and the lead compound decreased tau phosphorylation in recombinant cell lines expressing mutant tau and in neuron-differentiated iPSCs also in the absence of HSV-1 infection.

Conclusions: We conclude that MIF is a cellular host factor involved in HSV-1 replication and a drug target with antiviral efficacy. At the same time, MIF also plays a role in tau phosphorylation and is enriched in an oxidized conformation in brains of AD patients. MIF thus presents as a molecular link connecting HSV-1 infection and cellular pathology characteristic of neurodegenerative diseases involving aberrant tau phosphorylation.
AN Aβ42 DOUBLE MUTANT INHIBITS Aβ42-INDUCED PLASMA AND MITOCHONDRIAL MEMBRANE DISRUPTION IN ARTIFICIAL MEMBRANES, ISOLATED ORGANS AND INTACT CELLS

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Aims: Destabilization of plasma and inner mitochondrial membranes by extra- and intracellular amyloid β peptide (Aβ42) aggregates may lead to dysregulated calcium flux through the plasma membrane, mitochondrial-mediated apoptosis and neuronal cell death in patients with Alzheimer's disease.

Methods: In the current study, experiments performed with artificial membranes, isolated mitochondria, and neuronal cells allowed us to understand the mechanism by which a non-aggregating Aβ42 double mutant (designated Aβ42DM) exerts its neuro-protective effects.

Results: Specifically, we showed that Aβ42DM protected neuronal cells from Aβ42-induced accumulation of toxic intracellular levels of calcium and from apoptosis. Aβ42DM also inhibited Aβ42-induced mitochondrial membrane potential depolarization in the cells and abolished the Aβ42-mediated decrease in cytochrome C oxidase activity in purified mitochondrial particles. These results can be explained in terms of the amelioration by Aβ42DM of Aβ42-mediated changes in membrane fluidity in DOPC and cardiolipin/DOPC phospholipid vesicles, mimicking plasma and mitochondrial membranes, respectively. These observations are also in agreement with the inhibition by Aβ42DM of phospholipid-induced conformational changes in Aβ42 and with the fact that, unlike Aβ42, the Aβ42–Aβ42DM complex could not permeate into cells, but instead remained attached to the cell membrane. Although most of the Aβ42DM molecules were localized on the cell membrane, some penetrated into the cytosol in an Aβ42-independent process, and, unlike Aβ42, did not form intracellular inclusion bodies.

Conclusions: Overall, we provide a mechanistic explanation for the inhibitory activity of Aβ42DM against Aβ42-induced membrane permeability and cell toxicity and provide confirmatory evidence for its protective function in neuronal cells.
Aims: Microglia-based targets have received increased attention in neurodegenerative drug development. Among them, triggering receptor expressed on myeloid cells 2 (TREM2), a receptor on microglia, has been shown to be important for activating homeostatic microglia into disease-associated state that displays neuroprotective features in Alzheimer’s disease. Here, we developed a quantitative systems pharmacology (QSP) to explore TREM2 modulation therapies.

Methods: We developed a model of amyloid-ApoE dynamics and microglial activation upon TREM2 stimulation with key microglial and neuronal biomarkers based on pre-clinical data. The model was used to explore two mechanisms of TREM2 modulation, i.e., direct activation or block TREM2 shedding. The model also takes into account the microglial phenotype dynamics [1], and how this would influence the response.

Results: The model predicted that both mechanisms are comparable, but that blocking shedding is more efficient than directly activating TREM2 in obtaining the maximum pharmacodynamic effect on amyloid load reduction. We also predicted that the response depends on the microglial phenotype fractions present at the time of treatment, it can accelerate ramified to hypertrophic state, prolong and prevent it from transitioning into the dystrophic state.

Conclusions: As far as we are aware, our QSP neuroinflammation platform model is the first of its kind for application in drug discovery. The human version of this model is currently under development to guide translational research and support clinical trial design. The model can also be used to evaluate potential synergistic combination therapeutics, for example, TREM2-modulating antibodies with amyloid-targeted therapies. References [1] Sarma et al., ASCPT 2022.
OVERCOMING TREM2 ALZHEIMER’S DISEASE RISK VARIANT DEFICITS IN MICROGLIA WITH TREM2-TARGETED ANTIBODIES

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Aims: The microglial-expressed triggering receptor expressed on myeloid cells 2 (TREM2) plays a central role in Alzheimer’s disease (AD) pathology. The heterozygous R47H variant of TREM2 expresses as a loss of function and confers a similar risk for late-onset AD to that of the APOE ε4 allele variant. Based on this genetic evidence, therapeutic TREM2 activation has the potential to boost microglial responses to disease pathology. Here, we investigate the ability of TREM2 agonistic antibodies (Abs) to rescue microglial deficits associated with the R47H variant.

Methods: Given that the R47H variant impairs TREM2 splicing and mRNA and protein expression in mice but not in humans, human induced pluripotent stem cell-derived microglia (iPS-Mg) offer a more disease relevant model¹,². Using iPS-Mg expressing TREM2 common variant, patient R47Hhet, R47Hhom/isogenic and TREM2 knockout lines, we assessed the ability of the Abs to stabilise TREM2 at the cell membrane, enhance TREM2 signalling and stimulate microglial responses.

Results: Preliminary data indicate that TREM2 engagement by Abs result in TREM2 shedding inhibition in iPS-Mg and modulate TREM2-mediated microglial responses.

Conclusions: These findings recognise TREM2 activating antibodies as a promising therapeutic strategy for neurodegenerative diseases such as late-onset AD. Funded by: AstraZeneca

Aims: Several risk factors for late-onset Alzheimer’s disease (LOAD) have been identified that are highly expressed in innate immune cells, including CD33, TREM2, TREM1, and SPI1. Our group and others have found that the CD33, TREM1, and SPI1 risk alleles are associated with alterations of TREM1 and TREM2 expression, such that decreased TREM1 expression and a decrease in the TREM1:TREM2 ratio are associated with LOAD risk. The aim of this study is to clarify the effect of these risk alleles on TREM1 expression in the context of LOAD pathology.

Methods: Monocytes were isolated from individuals with risk alleles for SPI1 (rs1057233), CD33 (rs3865444), or TREM1(rs6910730) and stressed with aggregated amyloid beta (Aβ).

Results: Aβ stress induced TREM1 expression in monocytes carrying the CD33AA protective allele but not in those with the CD33CC risk variant. CD33CC monocytes also demonstrated impaired Aβ uptake following Aβ stress. However, when CD33CC monocytes were treated with a TREM1 agonist following Aβ stress, Aβ uptake was rescued and apoptosis was decreased. Similarly, TREM1 knockdown in CD33AA cells resulted in increased proapoptotic BAX expression. SPI1 encodes the transcription factor PU.1, a negative regulator of TREM1. While the SPI1 risk variant increases PU.1 expression, the SPI1 protective variant increased TREM1 expression following Aβ stress, as did the TREM1 protective variant.

Conclusions: These findings demonstrate that the CD33, SPI1, and TREM1 risk alleles converge to reduce TREM1 expression, and the protective effects of TREM1 in the context of Aβ stress. Ongoing work aims to understand mechanisms underlying the protective effect of TREM1 in LOAD.
NEUROPILINS: NOVEL RECEPTORS FOR THE VGF-DERIVED PEPTIDE TLQP-21

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Aims: VGF is a neuronal and neuroendocrine precursor protein, recently identified as a key driver of Alzheimer’s disease (AD) pathology and progression. Its proteolytic processing gives rise to several biologically active peptides, including TLQP-21. TLQP-21 regulates microglial function through its interaction with C3aR1 and C1qBP receptors. This project aims to discover novel binding partners for TLQP-21, investigate their biological significance and thus broaden our understanding of its biology and role in AD.

Methods: Recombinant proteins were produced in E.coli, and biotin-labelled version of TLQP-21 (Bio-TLQP21) was obtained from LifeTein. Screening for new TLQP-21 binding partners was performed using the Retrogenix Cell Microarray technology. Protein interactions were further validated using bio-layer interferometry, and X-ray crystallography is being used to obtain high resolution structures of protein-peptide complexes. In vitro assays are being performed to investigate the effect of the observed novel interactions on cell migration.

Results: Binding of Bio-TLQP21 was screened against 5,462 human plasma membrane proteins and cell surface-tethered secreted proteins, as well as 371 heterodimers expressed in HEK293 cells. Specific interaction was identified with the known binder C1qBP. Interactions were also observed with multiple isoforms and heterodimer combinations of Neuropilin 1 and 2 (NRP1; NRP2). The novel binding of TLQP-21 to NRP1 and NRP2 was further validated using bio-layer interferometry.

Conclusions: Cell microarray screen identified NRP1 and NRP2 as new binding partners for TLQP-21. Further characterisation of this interaction and understanding its cellular consequences are ongoing and may inform novel therapeutic strategies.
INCREASED RESPONSE OF PRIMARY ADULT MICROGLIA FROM 5XFAD MICE TO PRO-INFLAMMATORY STIMULI

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Aims: The importance of microglia in neurodegenerative diseases is well-known and these cells are therefore frequently used as a target for new pharmacological interventions. To study this cell type, isolation of early postnatal microglia from mice is a great tool but does not properly reflect conditions in aged or diseased individuals. Isolation of viable microglia from adult mouse brains of specific disease models via Magnetic Cell Sorting (MACS) opens new possibilities to assess the efficacy of microglia targeting treatments in vitro. Here we investigated the in vitro response of isolated adult microglia from 5xFAD mice to various stimuli in comparison to age matched non-transgenic (ntg) microglia.

Methods: Microglia were isolated from 9-months old 5xFAD mice and ntg littermates. Cultivated cells were stimulated with LPS or Aβ1-42 in presence or absence of anti-inflammatory agents (dexamethasone, Ibudilast, MCC950) and cytokine release (TNF-α, IL-6, IL-1β and KC/GRO) into the supernatant was measured.

Results: A 4-fold higher yield of microglia was obtained from 5xFAD brains compared to ntg littermates, reflecting the inflammatory status of 5xFAD brains. The response to pro-inflammatory stimuli was tremendously stronger in 5xFAD microglia revealing 10-fold higher levels of secreted cytokines compared to ntg microglia stimulated with the same stressor. Treatment of LPS-stimulated 5xFAD microglia with anti-inflammatory agents could significantly attenuate the cytokine release compared to the LPS-stimulated control.

Conclusions: Generation of a pure microglial fraction from adult brains of transgenic mice opens a variety of new possibilities to assess the efficacy of treatments in diseased microglia.
MODULATION OF MICROGLIA FUNCTION VIA OMEGA-3 POLYUNSATURATED FATTY ACIDS IN THE CONTEXT OF ALZHEIMER’S DISEASE

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Aims: Genome-wide association studies identified several genes, implemented in microglia phagocytosis, which are associated with an increased risk for Alzheimer’s disease (AD), offering microglia as potential therapeutic targets. Factors circulating in the blood, like food metabolites, might have effects on microglia function and thereby influence cognitive degeneration. Along this line, we investigated the effect of blood serum of AD patients and participants with cognitive decline on microglia by using an in vitro parabiosis system.

Methods: Microglia were incubated with human serum, encountered with pH-sensitive fluorescent particles and subsequently measured by flow cytometry. The University Hospital Graz provided us with serum from AD patients and age-matched controls (n = 30 per group). A second data set was obtained through a case-control study conducted by the EU D-CogPlast project, consisting not only out of blood samples taken before participants showed signs of cognitive deficit but also data on nutritional status (n = 209 per group).

Results: The serum of AD patients led to elevated phagocytosis levels, which were associated with cognitive impairment. To investigate a potential prognostic significance of the phagocytosis assay we made use of the serum from the D-CogPlast study. Here, we found no difference between the groups, but phagocytosis correlated negatively with the amount of eicosapentaenoic acid (EPA), one of the main omega-3 polyunsaturated fatty acids, in the blood.

Conclusions: EPA likely has direct and/or indirect effects on microglial phagocytic activity, which would offer a potential attenuation of neurodegenerative diseases by influencing protein deposition and microglia driven neuroinflammation.
POSTERS

SODIUM CHANNELS AND G-PROTEIN-COUPLED RECEPTOR GI SIGNALING AS OPPOSING MECHANISMS IN MICROGLIAL ACTIVATION

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Aims: Environmental toxins represent a potential risk factor for Alzheimer’s disease (AD) and have the potential to stimulate neuroinflammation. Sodium channels present on microglia represent an understudied target which may influence microglial activation. Similarly, the exact nature of Gi inhibitory signaling via G-protein coupled receptors present on microglia has not been fully explored. We here tested the hypothesis that low-level exposure environmental toxins which act on sodium channels (such as the pyrethroid permethrin) may act to prime microglia to be more responsive to other inflammatory factors such as stress. We then sought to determine if chemogenic inhibition via Gi signaling could block microglial priming.

Methods: Mice were chronically exposed to permethrin or vehicle for 14 days followed by 7 days of unpredictable stress or gentle handling and behavioral testing. Microglial activation was analyzed via immunofluorescent staining and sholl analysis. Next we utilized transgenic mice expressing hM4Di x CX3CR1 creER to enable expression of hM4Di on microglia. We then utilized the ligand JHU37160 to chemogenically inhibit microglia.

Results: Permethrin exposure followed by mild stress was associated with behavioral changes and microglial activation in the hippocampus. Treatment with JHU37160 in hM4Di x CX3CR1 creER mice blocked behavioral changes associated with permethrin and stress exposure.

Conclusions: Low level exposure to pyrethroid environmental toxins represents a potential risk factor for AD, which may prime microglia to be more responsive to other inflammatory factors such as stress. Additionally, targeting Gi inhibitory G-protein signaling in microglia may represent a therapeutic strategy to mitigate disease risk.
GENETIC LOSS OF TSPO DELAYS AND REDUCES THE PROGRESSION OF AMYLOID, TAU, INFLAMMATION AND TAU-ASSOCIATED BEHAVIORAL DYSFUNCTIONS.

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Aims: An increased expression of the 18kDa translocator protein (TSPO) is well demonstrated in Alzheimer’s Disease (AD). Its cellular origin mainly concerns glial cells, and the dynamics of TSPO over-expression showed an earlier involvement of astrocytes than microglia. However, the pathophysiological role of TSPO remains very poorly understood. In this study, we wanted to define the impact of TSPO inhibition on the appearance and the progression of AD markers.

Methods: WT, TSPO⁻/⁻, 3xTgAD and 3xTgAD;TSPO⁻/⁻ were used. AD-associated behaviors were measured, and neuropathological hallmarks and glial reactivity were quantified in different sub-regions of the hippocampus.

Results: Inhibition of TSPO in 3xTgAD mice induced a delay of the onset and a reduction of hippocampal levels of poorly and highly aggregated Tau (-44% and -82%, respectively) and Ab42 (-25% and -95%, respectively) forms at 9 months of age. Although the microglial IBA1 marker did not show any difference, the density of GFAP was reduced in 3xTgAD;TSPO⁻/⁻ mice with a reduction in the number of ramifications and size of astrocytes in the dorso-dorsal hippocampus and the hilus, suggesting a reduction in astrocyte reactivity. Key proteins involved in Ab metabolism were unaffected in 3xTgAD;TSPO⁻/⁻. Interestingly, the cognitive consequences of Tau over-expression in the hippocampus (mediated by adeno-associated virus injection) were also reduced in TSPO⁻/⁻ mice.

Conclusions: These data clearly demonstrate an early and damaging role of TSPO in the AD pathophysiology. Its genetic absence reduces neurochemical and behavioral AD markers and reduces astrocyte reactivity, suggesting that TSPO and astrocytes could be early therapeutic targets to treat AD.
NEUROD1 TRANSDIFFERENTIATES REACTIVE ASTROCYTES INTO MATURE NEURONS AND IMPROVES PATHOLOGICAL READOUTS IN THE TGAD-F344 MODEL OF ALZHEIMER’S DISEASE

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Aims: Neurodegeneration and neuroinflammation are hallmarks of Alzheimer’s disease (AD); goals for therapeutics aim to halt neuronal loss, increase neurogenesis, decrease reactive gliosis, and decrease neuroinflammation. Cellular reprogramming of somatic cells offers a potential targeted approach for modulating the neuronal loss in AD. Viral vectors allow in vivo delivery of transcription factors to induce transdifferentiation of astrocytes into specific subpopulations of neurons without an intermediate proliferative pluripotent stem cell phase. The conversion of reactive astrocytes into neurons is anticipated to increase the number of mature functional neurons.

Methods: The NeuroD1 transcription factor was used to induce transdifferentiation of reactive astrocytes into neurons in the hilus of the hippocampus in 14-month-old TgAD-F344 rats. The TgAD-F344 rat model recapitulates the amyloid accumulation, neuronal loss, reactive gliosis, and cognitive impairments found in AD. An adeno-associated virus (AAV2/5) containing the GFAP promoter +/- the NeuroD1 transcription factor was injected unilaterally into Tg and NTg rats. Electrophysiology or histopathology was performed 7 weeks post-injection.

Results: Histopathological analyses showed an increased number of NeuN-positive cells in the injected hemisphere, increased plaque-associated microglia (Iba1+) and decreased amyloid plaques (4G8) in the injected hemisphere of Tg animals in comparison to the uninjected hemisphere. Electrophysiological analyses determined the functional status of the new neurons within the hippocampal network.

Conclusions: Exploiting the presence of reactive astrocytes and converting them to functional mature neurons offers a potential targeted approach for modulating neuronal loss and improving the network imbalances in Alzheimer’s disease.
INFLUENCE OF EPIGALLOCATECHIN GALLATE STABILIZED GOLD NANOPARTICLES AMYLOID BETA FIBRIL INHIBITION

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Aims: A pathological Hallmark of Alzheimer's Disease (AD) is the significant deposition of amyloid beta (Aβ) plaques in the brain [1].

Methods: Epigallocatechin gallate (EGCG stabilized gold nanoparticle (AuNps) were synthesized as a Aβ inhibitor. The synthesized EGCG- AuNps characterized using UV-Vis Spectroscopy, DLS, XRD, SEM (EDAX) TEM (SAED)

Results: The synthesized a AuNps as functional therapeutic agent for AD.

Conclusions: A pathological Hallmark of Alzheimer's Disease (AD) is the significant deposition of amyloid beta (Aβ) plaques in the brain [1]. In recent years, natural compound (photochemical) have been largely used to study and identified as promising agents for the prevention and treatment of neurodegenerative diseases including AD [3]. Gold nanoparticle, with different size and shapes enable their multifunctional properties used in biomedical applications. Gold nanoparticle with appropriate surface conjugates with higher affinity has been studied and acknowledges the interaction between Bio Nano interfaces [4]. The surface covering or anchoring conjugates will play a major role in vitro and in Vivo. Herein, We reported Epigallocatechin gallate (EGCG stabilized gold nanoparticle (AuNps) were synthesized as a Aβ inhibitor. The synthesized EGCG-AuNps characterized using UV-Vis Spectroscopy, DLS, XRD, SEM (EDAX) and TEM (SAED) and the biocompatibility were studied by using MTT and scratch assay. The synthesized EGCG-AuNps inhibiting Aβ aggregation, dissociation Aβ fibrils which lead to prevent plaque formation, which confirmed using Thioflavin T- assay and TEM studies. Thus, this work provides new insights into the synthesized a AuNps as functional therapeutic agent for AD.
A LOGICAL NETWORK-BASED DRUG-SCREENING PLATFORM FOR ALZHEIMER’S DISEASE REPRESENTING PATHOLOGICAL FEATURES OF HUMAN BRAIN ORGANOIDS

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Aims: Developing effective drugs for Alzheimer’s disease (AD), the most common cause of dementia, has been difficult because of complicated pathogenesis. Here, we report an efficient, network-based drug-screening platform developed by integrating mathematical modelling and the pathological features of AD with human iPSC-derived cerebral organoids (iCOs), including CRISPR-Cas9-edited isogenic lines.

Methods: We use 1,300 organoids from 11 participants to build a high-content screening (HCS) system and test blood-brain barrier-permeable FDA-approved drugs. Mass production of iCOs that are uniform in size and homogeneous in cell composition enabled us to perform drug screening using HCS system on a physiologically relevant platform. Mathematical modelling considering a network of molecular pathways and relevant genetic factors was employed to identify several FDA-approved drugs as candidates for drug repositioning.

Results: Our iCOs clearly show that amyloid-beta aggregates are formed in extracellular interstitial spaces, and hyper-phosphorylated tau co-localizes intracellularly along with neuronal marker MAP2. This could be an appropriate model reflecting characteristics of the actual disease-related human brain lesions. Next, we developed a relevant mathematical model and could propose candidate drugs. Finally, we treated the iCOs with the selected drugs and monitored the levels of Aβ or tau deposition and found that all of the candidate drugs were effective to some degree in reducing Aβ or tau deposition.

Conclusions: Our network-based drug-screening platform by integrating mathematical modelling and pathological traits of human iCOs was successfully validated. We thus herein introduce a reliable strategy that could enable precision medicine by engaging the convergence of mathematical modelling and pathological features of brain organoids.
MULTIPLE NEURODEGENERATIVE PATHOLOGIES IN AN ALZHEIMER DISEASE PATIENT TREATED WITH FORNICAL DBS

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Aims: To evaluation of the true benefit and risk potential of deep brain stimulation (DBS) on slowing cognitive decline in AD patients.

Methods: A 72-year-old woman initially presented with mild cognitive impairment in 2006. In 2008, she became noticeably less verbal and began to experience progressive memory impairment, difficulty making decisions, and mood instability. In 2012, she entered into the DBS-f trial for AD. In 2018, she became septic due to a urinary tract infection and died.

Results: The autopsy confirmed multiple proteinopathies including AD related change, diffuse neocortical Lewy body disease, TDP-43 proteinopathy and a nonspecific tauopathy.

Conclusions: It is most remarkable that this patient’s neurodegeneration involved at least three: Alzheimer’s disease typified by neurofibrillary tangles and neuritic plaques, Dementia with Lewy Bodies, and either LATE-NC or FTLD-TDP43 (or both). Notably, the pathological changes associated with these mechanisms were relatively advanced. It is possible that there are other non-AD pathologies waiting to be discovered, and sobering to note how many non-AD pathologies were present in this participant who presented clinically with AD. It is possible that this overlap of mechanisms is coincidental. However, a question also arises that whether DBS could contribute in an unknown way to one or more of these mechanisms of neurodegeneration. Such an effect of DBS has not been reported in autopsy studies of DBS for Parkinson’s disease, and has not been observed in animal models treated with DBS. Future studies of DBS for AD will need to take these possible safety concerns into consideration.
CHRONIC INTRANASAL OXYTOCIN TREATMENT RESTORES SOCIAL MEMORY LOSS IN THE APPSWE/PS1DE9 MOUSE MODEL OF ALZHEIMER’S DISEASE

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Aims: Alzheimer’s disease, the main cause of dementia in the elderly, is accompanied by social memory deficits. Oxytocin is a pro-social neuropeptide that has recently been shown to be neuroprotective. Here, we characterized oxytocin production in the APPswe/PS1DE9 mouse model of Alzheimer’s disease, and evaluated the potential of oxytocin to correct social memory impairments in those mice.

Methods: We used qPCR to quantify oxytocin mRNA levels in the hypothalamus of 12 month old male mice. We developed and validated a protocol to deliver oxytocin via intranasal in mice and implemented this protocol to evaluate the consequences of oxytocin on social memory in APPswe/PS1DE9 using the 5-trial social task.

Results: We observed a decrease trend in the levels of oxytocin in APPswe/PS1DE9 compared to wild type mice. We implemented a validated intranasal oxytocin delivery protocol to increase oxytocin cerebral levels and evaluate the consequences of oxytocin on social memory in APPswe/PS1DE9. We observed that APPswe/PS1DE9 treated with oxytocin were protected against social memory loss, which was present in APPswe/PS1DE9 treated with vehicle

Conclusions: Together, our results suggest that oxytocin could be a potential therapeutic approach to correct social memory deficits in Alzheimer’s disease.
Aims: Preclinical studies have suggested that low-intensity transcranial focused ultrasound (tFUS) may have therapeutic potential for Alzheimer’s disease (AD) by opening the blood-brain barrier (BBB), reducing amyloid pathology, and improving cognition. However, its efficacy and safety remain to be elucidated in humans. This study investigated effects of tFUS on BBB opening, regional cerebral metabolic rate of glucose (rCMRglu), and cognitive function in AD patients.

Methods: Eight patients with AD received image-guided tFUS stimulation to the right hippocampus with intravenous injection of microbubble ultrasound contrast agents. Patients underwent brain magnetic resonance imaging (MRI), $^{18}$F-fluoro-2-deoxyglucose positron emission tomography (PET), and neuropsychological assessments before and after the sonication.

Results: No evidence of transient BBB opening was found on T1 dynamic contrast-enhanced MRI. However, immediate recall ($p = 0.03$) and recognition memory ($p = 0.02$) were significantly improved on the verbal learning test. PET image analysis demonstrated increased rCMRglu in the right hippocampus ($p = 0.001$). In addition, increases of hippocampal rCMRglu was correlated with better performance in recognition memory (Spearman's $\rho = 0.77$, $p = 0.02$). No adverse events were observed.

Conclusions: Our results suggest that tFUS may have beneficial effects on brain function and cognition in AD patients, even without BBB opening. Further larger studies are warranted to explore and optimize sonication protocols and to confirm the efficacy and safety of tFUS in AD.
LICOCHALCONE A ENHANCES COGNITIVE FUNCTION BY ENHANCING MITOCHONDRIAL BIOGENESIS AND DECREASING Aβ IN A NEUROINFLAMMATORY MOUSE MODEL

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Aims: Mitochondrial biogenesis (MB) plays a major role in the pathogenesis of Alzheimer's disease (AD). Licochalcone-A (LCA); a flavonoid found in Glycyrrhiza, was recently found to be a positive regulator of MB in a spinocerebellar-ataxia3 model through up-regulating PGC-1α. Previous work by our group could demonstrate memory enhancement in a neuroinflammatory mouse model. Moreover, we were able to link MB to LCA's mechanism of action in AD, by an upregulation in PGC-1α. Interestingly, Aβ; one of the major hallmarks of AD, was found to be linked to mitochondrial dysfunction where Aβ impairs the Electron transport chain (ETC) and mitochondrial enzymes. Accordingly, the main goal of this study was to further examine the effect of LCA on MB and function, in addition to testing the involvement of Aβ in its mechanism of action.

Methods: This was achieved by quantifying the genetic expression of BACE-1 as well as PGC-1α downstream genes, starting with NRF-1; a key regulator in the MB pathway, followed by ND-1, a downstream gene and a subunit of complex-I of the ETC. Furthermore, ATP and Aβ levels were measured using ELISA in harvested mice brains.

Results: LCA, which improved memory and cognition in our neuroinflammatory mouse model, was able to increase the expression levels of NRF-1 and ND-1. Mitochondrial function was accordingly improved and this was noticed in the increased brain ATP levels. Moreover, LCA downregulated the BACE-1 enzyme responsible for Aβ synthesis, which was confirmed by a decrease in Aβ levels.

Conclusions: LCA displays neuroprotection through exerting MB-enhancing and anti-amyloidogenic activities.
THE ROLE OF SEX HORMONE THERAPIES ON COGNITIVE PERFORMANCE AT THE PRECLINICAL ALZHEIMER’S DISEASE STAGE: AN OBSERVATIONAL STUDY

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Aims: The neuroprotective efficacy of sex hormones, particularly oestrogen, is widely implicated to mitigate against cognitive decline and potentially delaying Alzheimer’s disease (AD) onset. However, use of oestrogen-based Hormone Replacement Therapies (HRT) for protection from cognitive decline remains inconclusive. The objective of this study is to investigate the role of HRT on cognition in cognitively healthy females and observe the moderating capacity in groups stratified by AD-risk and Apolipoprotein-Ɛ4 (APOE-Ɛ4) status.

Methods: Retrospective analysis of CHARIOT:PRO main study (987 preclinical adults: 60-85 years, screened at Imperial College London) was performed. 416 females were stratified depending on their HRT usage, among their APOE-Ɛ4 status and AD-risk through a novel AD-risk status classification method. Neuropsychological performance scores assessed cognitive status across multiple domains, through administration of the Repeatable Battery Assessment of Neuropsychological status.

Results: Preliminary results indicated that HRT usage in females had a favourable effect on language performance (p=0.027) and global cognition (p=0.025) compared to those who didn’t report HRT usage. Females who either didn’t possess the APOE-Ɛ4 allele (p=0.033) or had a low-risk of AD development (p=0.010) experienced the strongest beneficial cognitive effects of HRT on global cognition.

Conclusions: Study results further substantiate literature suggesting HRT improves cognitive performance in females. Specifically, data from this observational study indicates a novel interaction between AD risk-status at point of HRT initiation and proposed beneficial effect of HRT on cognition. Importantly, data suggests that HRT could be favourable in low-risk individuals, providing a potential prophylactic role to reduce AD incidence and offer cognitive protection.
NEUROPROTECTIVE PROPERTIES OF EXTRACTS FROM EUCALYPTUS GLOBULUS BIOMASS AND ITS BENEFICIAL EFFECTS IN AN ALZHEIMER’S DISEASE MOUSE MODEL

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**Aims:** This study aimed to evaluate the in vitro and in vivo protective effect of extracts obtained from E. globulus biomass. For that, a cytotoxicity screening was performed in several cell lines considering a future administration, the neuroprotective and anti-inflammatory properties, as well as BBB permeability were evaluated. Finally, the beneficial effects on AD-like pathology and behaviour were analysed in an AD mouse model.

**Methods:** Cytotoxicity was assessed by MTT assay. The Aβ levels were investigated by ELISA and the anti-inflammatory effect was analysed by NO assay and RT-PCR. ROS production and mitochondrial membrane potential were assessed by Amplex Red and TMRE assays, respectively. BBB permeability was studied by PAMPA assay. The effect in anxiety and cognitive deficits after intranasal administration to APP/PS1 mice was evaluated by open field and fear conditioning tests.

**Results:** The extracts revealed anti-inflammatory effect in LPS- and Aβ-stimulated microglia, attenuated Aβ-induced neuronal death, restored mitochondrial membrane potential and diminished Aβ secretion and ROS production in N2A-APPswe cells. One of the extracts demonstrated to cross the BBB and its intranasal administration to APP/PS1 mice decreased Aβ brain levels, reduced anxiety and showed a positive impact on amygdala-dependent memory.

**Conclusions:** These results suggest the beneficial effects of extracts obtained from E. globulus biomass on AD-like pathology and cognitive impairment that may be attributed to the ability to decrease Aβ production together with anti-inflammatory and antioxidant actions. Therefore, these extracts could be used as raw material to develop efficient and safe nutraceuticals and/or plant-based medicinal products useful for AD prevention or treatment.
CENTRALLY ACTING ANOREXIGENIC PEPTIDES AS A TREATMENT OF NEURODEGENERATION IN PRECLINICAL MODELS

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Aims: Objectives: Despite immense search for treatment of Alzheimer’s disease (AD), there is still no effective drug suppressing broad spectrum of AD hallmarks, such as β-amyloid (Aβ) plaques, Tau hyperphosphorylation, neuroinflammation or decreased neurogenesis. Recently, a link between obesity and/or type 2 diabetes and increased risk of AD development was described. Therefore, anorexigenic compounds with glucose-lowering properties, such as glucagon-like peptide-1 analog liraglutide, and prolactin-releasing peptide (PrRP) were repurposed as possible neuroprotective compounds.

Methods: Liraglutide and palmitoylated PrRP analogs (palm¹-PrRP and palm¹¹-PrRP), acting centrally after peripheral administration, were examined in three mouse models of neurodegeneration for their potential beneficial effects. Spatial memory changes were monitored in Y-maze test. The histopathological changes were measured using Western blot or immunohistochemistry.

Results: Firstly, mice with obesity and prediabetes induced by monosodium glutamate (MSG) with increased phospho-rylation of Tau protein in the hippocampi were studied. 14-day-long subcutaneous (SC) treatment with palm¹-PrRP resulted in increased activation of insulin signaling and attenuated Tau phosphorylation in hippocampi. Secondly, the effect of palm¹¹-PrRP was tested in THY-Tau22 mice overexpressing mutated human Tau protein, a model of Tau pathology. Two months of SC treatment improved short-term spatial memory, attenuated Tau phosphorylation and increased synaptogenesis. Finally, double transgenic APP/PS1 mice, a model of Aβ pathology, were treated with palm¹¹-PrRP31 for 2 months. Palm¹¹-PrRP31 treatment reduced the Aβ plaque load in the hippocampus, significantly reduced hippocampal microgliosis and cortical astrocytosis and increased neurogenesis in the hippocampus.

Conclusions: Conclusion: Anorexigenic peptides liraglutide and palmitoylated PrRP have beneficial neuroprotective properties targeting the main hallmarks of AD.
INVESTIGATION OF BIOCHEMICAL ALTERATIONS IN ALZHEIMER’S DISEASE TRANSGENIC MOUSE MODEL AFTER ISCHEMIC STROKE USING FTIR IMAGING SPECTROSCOPY

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Aims: Alzheimer’s disease (AD) is the most common dementia and is associated with neuronal death leading to cognitive dysfunctions. Stroke is one of the major risk factors for dementia; up to 30% of stroke patients develop cognitive dysfunction within 3 years. However, the molecular mechanisms of neuronal damage and glial responses following stroke, which can lead to cognitive decline are not fully elucidated. Thus, treatments of these long-term consequences of stroke are hampered by gaps in understanding molecular mechanisms related to neurodegenerative processes, such as damage of myelination and amyloid protein aggregation. This study is aimed to investigate molecular changes of proteins and lipids within the brain area affected by stroke in an AD transgenic mouse model. Expected results might help to find new strategies for neuroprotection and restoration following stroke.

Methods: Ischemic stroke was induced by permanent middle cerebral artery occlusion by electrocoagulation surgery stroke in AD transgenic mice and healthy controls. To reveal molecular changes of the secondary structure of proteins and lipids, the brain sections were analyzed by Fourier Transform Infrared Spectroscopy (FTIR). The study was complemented with inductively coupled plasma mass spectrometry (ICP-MS) and immunolabelling.

Results: Our FTIR results revealed that already after 3 hours post-stroke occurs significant changes in the secondary structure of lipids like lipid oxidation and cell membrane functions in the AD transgenic mice that precede protein aggregation.

Conclusions: Changes in the secondary structure of lipids occur before structural changes in proteins, which may result in further neuronal damage and activation of a detrimental glial response.
Aims: Until now many methods were tried to stimulate brain tissue to treat several neurological diseases. A recently developed Transcranial Pulse Stimulation device with NEUROLITH® provide more precise targeting and arranging intensity of sound waves/shock waves. TPS has ability to reach deep target tissue due to conductivity effects. Here we want to share our experiences.

Methods: We applied the treatment procedure of six sessions to 20 patients. During this treatment procedure we kept their drug regime as they were using before. We applied CERAD and MMSE tests before the treatment and 4 weeks after 6 sessions of treatment. During each session, we gave 6000 of pulse stimulations to the entire cortex with the navigation system of NEUROLITH®.

Results: We compare the the results of tests and clinical observations of ours and care givers after all. We haven’t seen any side effect. We observed beneficial effects of TPS application in all of them with various degrees. All of the patients become more social and interactive with their family members.

Conclusions: Transcranial Pulse Stimulation (TPS) is a revolutionary treatment option for neurodegenerative diseases. Our study showed promising therapeutic effects. We need further placebo controlled studies of big cohorts.
EMORY-SAGE-SGC TREAT-AD CENTER: DEVELOPING TOOLS FOR NEW MEDICINES IN ALZHEIMER’S DISEASE

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Aims: Objectives: The Emory-Sage-SGC TREAT-AD Center aims to diversify the Alzheimer’s disease (AD) target pipeline by promoting the evaluation of under-studied AD targets. To this aim, we are generating and openly distributing validated experimental tools necessary to test target predictions generated through systems biology study of human disease.

Methods: Method: The center uses integrated systems biology approaches to discover new targets from a set of prioritized therapeutic hypotheses. Targets are then evaluated for target enablement opportunities. All TREAT-AD investigators place all data, knowledge, reagents, and tools into the open domain with no intellectual property claims.

Results: Nascent AD targets were mapped to 15 biological domains (BDs) and prioritized based on a TREAT-AD overall score, an unbiased bioinformatic assessment across multiple lines of evidence for overall AD-risk. Within these 15 BDs, we prioritized more than 30 targets for target enabling package (TEP) development. TEPs include expression constructs, knockout cell lines, assays, antibody validation, and crystal structures. All reagents are developed to meet established quality criteria. For a subset of tractable targets (MSN, SYK, SFRP1, and CAPN2), chemical probe development is underway to provide tools to test these therapeutic hypotheses in cellular and animal systems. All data and reagents will be publicly shared through standard repositories and cataloged on the AD Knowledge Portal site.

Conclusions: Conclusion: The open drug discovery approach of TREAT-AD is aimed to de-risk potential AD therapeutics to catalyze robust and independent evaluation of a diverse portfolio of promising yet untested AD therapeutic hypotheses.
POLYSOMNOGRAPHIC PREDICTORS FOR THE COGNITIVE DECLINE OF PATIENTS WITH MILD-MODERATE ALZHEIMER’S DISEASE AND OBSTRUCTIVE SLEEP APNEA.

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Aims: Given the contradictory findings on the impact of obstructive sleep apnea (OSA) over the cognition of Alzheimer’s disease patients, we aimed to unravel possible polysomnographic determinants for an increased cognitive decline after 12 months of follow-up.

Methods: Observational, prospective, single-center study, including consecutive patients diagnosed with mild-moderate Alzheimer’s disease (NCT02814045). The individuals were submitted to overnight polysomnography, followed by neuropsychological evaluations at baseline and after 12 months of follow-up. Only individuals with OSA (apnea-hypopnea index [AHI] ≥ 5) were included in the analyses.

Results: We evaluated 129 patients with a mean (SD) of 23.49 (2.21) points in the Mini-Mental State Examination at the baseline. The cohort was mostly composed of females (56.8%), with a mean age of 75.41 (5.30) years and an AHI of 29.08 (23.18). A selection process based on random forest revealed that the hypopnea index was the most important characteristic to predict the cognitive decline at the 12-month follow-up, followed by the AHI, arousals associated with periodic limb movements, and non-specific arousals.
Conclusions: Our preliminary findings unravel a set of polysomnographic characteristics that could predict an increased cognitive decline of mild-moderate Alzheimer’s disease patients after 12 months of follow-up, revealing possible features that could explain the discrepancy of findings related to the impact of OSA on Alzheimer’s disease. Further studies are warranted to investigate relationships of causality and underlying mechanisms.
WAVE-AD: WEARABLE NEUROMODULATION DEVICE FOR THE TREATMENT OF ALZHEIMER’S DISEASE

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**Aims:** The TNM™ Device, developed by Scion NeuroStim, LLC, delivers time-varying caloric vestibular stimulation (tvCVS) for the treatment of neurological indications. In a single site RCT, tvCVS treatments significantly and durably improved memory, attention, apathy, depression, anxiety and activities of daily living in a Parkinson’s disease population. Based on these and previous mechanistic findings, a pilot RCT with open label extension (NCT05032482) has been initiated to explore the safety and efficacy for treating mild late-onset Alzheimer’s Disease (LOAD). Primary: Collect evidence to evaluate the safety and efficacy of tvCVS for improving global cognition. Secondary: Evaluate the efficacy of tvCVS for improving function and providing clinically meaningful benefits. Exploratory: Explore the impact of tvCVS on treating neuropsychiatric symptoms, cognition (including executive function), caregiver burden and disease-related bloodborne biomarkers.

**Methods:** Participants will be randomized (1:1) to receive either tvCVS or passive treatments self-administered twice daily in the home setting over 24 weeks. During the OLE, all participants will receive tvCVS for 24 weeks.

**Results:** Data collection for the RCT is currently ongoing. This study builds on single site RCT data which showed that 8 weeks of twice-daily tvCVS treatments was associated with significant and durable gains in both performance-based measures and patient reported outcomes related to cognition in Parkinson’s patients with cognitive dysfunction.

**Conclusions:** These studies will provide critical data to evaluate tvCVS as a safe and effective treatment for AD and will guide the development efforts for a follow-on pivotal trial to definitively evaluate the safety and efficacy of TNM™ Device therapy for dementia in mild AD.
AMYLOID RESEARCH CONSULTANTS (ARC): A NOVEL APPROACH TO PHARACEUTICAL DEVELOPMENT FOR ALZHEIMER’S, PARKINSON’S, AND TYPE 2 DIABETES.

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Aims: Effective treatments for Alzheimer’s, Parkinson’s, and Type 2 Diabetes. An impossible dream? Maybe not. But achieving that dream may require identifying what to target and being able to visualize, isolate, synthesize, stabilize, and hit those targets without disrupting vital physiological processes. Past efforts have focused primarily on fibrils formed by amyloid beta, alpha synuclein, and amylin (IAPP); the most stable and visible hallmarks of these diseases. But recent findings indicate that some smaller assemblies, oligomers and transmembrane channels, are more toxic. ARC focuses on these.

Methods: We have developed detailed molecular models of both toxic and non-toxic assemblies of all three amyloids as they progress from trimers up to large annular protofibrils, lipoproteins, and channels composed of up to 64 monomers, plus how peptides such as humanin bind to these oligomers and reduce their toxicity. These symmetric β-barrel models are consistent with well established molecular modeling theory and methods and with a vast amount of low-resolution EM and other biophysical/biochemical data. In most cases all monomers of our models have identical structures.

Results: Yes, these models are hypothetical. But hypothesis-based research is a cornerstone of contemporary science.

Conclusions: Aspects of our models can be tested experimentally. They even suggest how to reduce disorder and polymorphism that have prevented determination of high-resolution structures of most amyloid oligomers. But perhaps most important, they suggest which portions of the oligomers and channels should be targeted for development of antibody and drug therapies and how to synthesize and stabilize these targets so effective therapeutics can be developed.
O-GLCNAcylation Alleviates the Pathological Features of Alzheimer’s Disease by Inhibiting Necroptosis

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Aims: O-linked β-N-acetylglucosaminylation (O-GlcNAcylation) is a post translational modification which is enriched in brain and several O-GlcNAcylated proteins, such as tau and α-synuclein, are associated with neurodegenerative diseases. It has been known that O-linked β-N-acetylglucosaminase (OGA) inhibition decreased the amount of Aβ plaques in the brain of AD mouse models. Necroptosis has been found to be activated in humans' brains with AD and is positively correlated with the pathological features like neuronal death, neuroinflammation. Inhibition of necroptosis in AD effectively suppresses neuroinflammation. In this study, we evaluated the association between O-GlcNAcylation and necroptosis in AD.

Methods: We evaluated the expression level of O-GlcNAcylation-related proteins, necroptosis-related proteins, and Aβ in human brain samples and mouse brain samples by western blotting and/or immunohistochemistry. Furthermore, we generated 5xFAD with OGA haploinsufficiency and evaluated the cognitive function by behavioral tests.

Results: Expression level of O-GlcNAc was decreased in both human brains with AD and 5xFAD brains. Also, the expression levels of necroptosis-related proteins were increased in both human brains with AD and 5xFAD brains. OGA haploinsufficiency increased O-GlcNAc levels in the brain and decreased activation of necroptosis, thereby reducing neuronal loss in the brain of 5xFAD. Furthermore, increased O-GlcNAc levels ameliorated cognitive deficits in 5xFAD mice and reduced Aβ accumulation.

Conclusions: Here, we found that O-GlcNAcylation plays a protective role in AD by inhibiting necroptosis. O-GlcNAcylation ameliorated AD pathology including Aβ plaques, neuronal loss, neuroinflammation and cognitive decline. Thus, our data establish the influence of O-GlcNAcylation on Aβ accumulation and neurodegeneration, suggesting O-GlcNAcylation-based treatments as potential interventions for AD.
THERAPEUTIC POTENTIAL OF SIRT2 INHIBITION BY THE COMPOUND 33I IN A TRANSGENIC MOUSE MODEL OF ALZHEIMER'S DISEASE

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Aims: Sirtuin 2 (SIRT2) is a deacetylase whose relevance has increased exponentially during the last years due to its potential regulatory role in metabolism and longevity. In addition, SIRT2 has been associated to aging and neuroinflammation, which are essential mechanisms for the progression of Alzheimer’s disease (AD). In this study, we analyzed the potential effect of SIRT2 inhibition by a specific inhibitor, the compound 33i, on a transgenic mouse model of AD, the APP/PS1 model.

Methods: 33i was administered intraperitoneally to 6-months-old APP/PS1 mice. At 8th week of treatment, memory was assessed by Morris Water Maze (MWM) and long-term potentiation (LTP) was measured in hippocampal slices. Moreover, neuroinflammation and amyloid pathology were analyzed by immunofluorescence and qPCR.

Results: 33i administration partially reverted cognitive impairment in MWM and restored LTP compared to APP/PS1-vehicle mice. These benefits were further confirmed at the molecular level where amyloid pathology and neuroinflammation were analyzed. SIRT2 inhibition reduced amyloid beta 1-42 levels and the number of amyloid plaques in APP/PS1 mice, an effect accompanied by a reduction in gene expression of inflammatory cytokines. Moreover, this study demonstrates that SIRT2 systemic inhibition is a safe treatment for peripheral metabolism, since no significant changes were observed in glucose or insulin tolerance tests.

Conclusions: These results establish a potential target and a promising molecule to treat AD. Moreover, they highlight the importance of further investigate the specific roles of SIRT2 in order to understand the pathophysiology of age-related neurodegenerative diseases, a key point to achieve new therapeutic strategies.
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POSTERS

THETA-TACS COMBINED WITH COGNITIVE TRAINING MODULATES ERPS WHILE IMPROVING PERFORMANCE IN AN ODDBALL TASK: A PILOT STUDY

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Aims: Despite great efforts in the last decades, Alzheimer’s Disease is a major sociosanitary problem. Cognitive training has proved to be useful, but only to a certain degree. On the other hand, transcranial alternate electrical stimulation (tACS) techniques are receiving attention in the last years, and seem to be able to act upon AD-related cognitive decline. In any case, recent reviews have pointed out that combined interventions as the used in this pilot study are a good choice for these patients, in order to improve cognitive performance.

Methods: Twenty-one volunteers with Mild Cognitive Impairment or Subjective Cognitive Decline diagnoses underwent 12 sessions of cognitive entrainment. During the last 6 sessions, they also received 20-minutes theta-tACS (6 Hz; n=12) or sham-tACS (n=9), applied on the scalp over the left dorsolateral prefrontal cortex (IDLPFC, F3 electrode position). In order to evaluate treatment efficacy, participants underwent two additional sessions (before and after treatment, pre-T and post-T sessions) in which they performed an oddball task while EEG was recorded.

Results: Statistical analysis comparing post-T session with pre-T session evidenced that only participants that received theta-tACS showed: 1) larger d’; and 2) lower P3b mean amplitudes.

Conclusions: These results seem to indicate that theta-tACS applied over the IDLPFC, in combination with cognitive entrainment, is able to modulate cognitive function in people in the AD continuum. This pilot study encourages the use of these combined interventions in larger samples, in order to seek for effective treatments with the aim of slowing down the progression of the disease.
STUDY OF SYNAPTIC-RELATED PROTEIN CHANGES ON A MURINE MODEL FOR ALZHEIMER’S DISEASE TREATED WITH A RXR AGONIST

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Aims: Alzheimer’s Disease (AD) is a neurodegenerative disorder that presents cognitive impairment and memory loss. Additionally, Retinoid X Receptors (RXR) are transcription factors activated by retinoids such as bexarotene, that has been reported to produce cognitive improvement on the triple transgenic Alzheimer’s Disease (3xTg-AD) mice model. According to that, our aim was to study the molecular mechanisms associated to the cognitive improvement on bexarotene treated 3xTg-AD mice; and additionally, to evaluate changes based on myelinating, synaptic, and AP-1 transcription factor related proteins.

Methods: Immunohistochemistry assays were performed using confocal microscopy analysis on brain coronal sections from very old 3xTg-AD mice treated with bexarotene (100 mg/kg) for 30 days. Then, they were compared to the wild-type littermates and the 3xTg-AD mice treated with the vehicle. The evaluated proteins were Myelin Basic Protein (MBP), FosB, the glutamate metabotropic receptor 5 (mGlur5) and SHANK1/2/3, on different hippocampal and cortical regions.

Results: Bexarotene partially recovered the MBP levels on CA1, CA3 and entorhinal cortex, in very old 3xTg-AD, and increases the co-labeling of MBP and FosB in mature oligodendrocytes. In addition, it also reduced the FosB, mGluR5 and SHANK1/2/3 levels on hippocampal and cortical regions.

Conclusions: Bexarotene treatment on 3xTg-AD mice partially recovered myelination and showed a role together with FosB on the oligodendrocyte proliferation and differentiation. By the other hand, reduction on the SHANK1/2/3 and mGluR5 levels could be associated with the protein mobilization and remodeling processes on the postsynaptic density, which also could partially explain previously reported LTP improvement in the model.
A-LIPOIC ACID HAS THE POTENTIAL TO NORMALIZE COPPER METABOLISM, WHICH IS DYSREGULATED IN ALZHEIMER’S DISEASE

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Aims: Copper metabolism is dysregulated in Alzheimer’s disease (AD), which may trigger the development of AD pathology. AD is accompanied by substantially elevated levels of copper in extracellular space and simultaneous copper deficiency in brain tissue. These changes result in decoppering of cellular copper proteins, which can hypothetically be detrimental to neurons. In the current study, we follow the widely accepted hypothesis that the normalization of copper metabolism leads to the prevention or slowing of the disease and search for new copper-regulating ligands, such as natural ligand α-lipoic acid (LA).

Methods: Human SH-SY5Y neuroblastoma cell line was used in all toxicity and copper redistribution experiments with ICP-MS. Experiments were done with three copper chelating agents: trientine (TETA), D-penicillamine (DPA) and LA. To understand more about LA effect on AD phenotype, two different Drosophila melanogaster AD lines were used - UAS-APP.Aβ42.D694N.VTR (Iowa flies) for negative geotaxis and UAS-APP695-N-myc, UAS-BACE1/TM6B (APP/BACE1 flies) for overexpression in the eye photoreceptors to study neurodegeneration.

Results: We showed that TETA and LA were not toxic to the cells in the presence of copper. Next, we showed that only LA could redistribute copper between extracellular and intracellular environments. Moreover, experiments with fruit flies showed that LA has the potential to prevent the development of AD phenotype.

Conclusions: In conclusion, we hypothesize that LA can avoid or inhibit the following processes in AD pathology: (1) an increase of extracellular copper levels during aging; (2) amyloidogenic proteolytic cleavage of APP into amyloid peptides; (3) demetallation of essential copper proteins.
POSTERS

LONG-TERM RESULTS OF ALZHEIMER’S AND PARKINSON’S DISEASE AFTER TRANSCRANIAL PULSE STIMULATION (TPS) OF THE BRAIN WITH FOCUSED EXTRACORPOREAL SHOCK WAVES (ESW).

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Aims: The introduction of an ESW generator with navigation system (Neurolith, Storz Medical) is a breakthrough for the precise treatment of brain structures. After a treatment series with TPS, Alzheimer’s patients showed after 3 months a significant functional gain in cognitive abilities and a repair of cortical areas responsible for memory functions. Now case histories will be presented with long-term observations of patients with Alzheimer’s and Parkinson’s disease as a critical endeavor for future treatments.

Methods: 6 patients with mild Alzheimer’s disease (Mini mental status >18) were treated initially 6 times during 2 weeks with TPS. After this, the patients received one TPS session monthly over 3 years. The results were assessed with the CERAD Plus score. 4 patients with Parkinson’s disease assessed with the Unified Parkinson Rating Scale received TPS after the aforementioned protocol but had every year a booster (6 sessions in 2 weeks).

Results: Alzheimer’s: After a steady enhancement of the cognitive abilities over 12 months of 12 %, the results diminished during the second year back to the baseline. A two weeks booster with TPS like the initial treatment enhanced the results again with 4.5 %. After 3 years the results were on the level of the natural history (Hallikainen 2013). Parkinson’s: From the beginning the symptoms improved by roughly 50% and remained stable until to date for over 4 years and in one patient for 8 years.

Conclusions: It is conceivable that more frequent testing and earlier boosters would also achieve a better result in Alzheimer’s patients.
Aims: Rice Bran Extract (RBE) was reported to enhance memory and cognition in a neuroinflammatory mouse model. One of the proposed mechanisms was through activation of peroxisome-proliferator-activated-receptor γ (PPARγ), as RBE components such as polyunsaturated fatty acids (PUFA) were previously reported to act on PPARγ receptors. Previously we were able to show that RBE enhances cognitive functions in mice and this effect is reversed using a PPARγ antagonist. In this study, we further assessed the effect of RBE administration on CD36 levels that is regulated by PPARγ. Moreover, we measured the levels of important PUFAs which are reported to affect cognition and/or PPARγ in the brains of RBE treated mice. Finally, since PPARγ has been shown to affect the levels of amyloid beta(Aβ), the level of Aβ1-42 was measured in brains of treated mice.

Methods: Mice were pre-treated orally with Egyptian RBE (100mg/kg) for 21 days and LPS was injected during the last week for inducing the neuroinflammatory mouse model. Brains of treated mice were used for measuring the expression levels of CD36 and Aβ1-42 protein. Moreover, DHA, EPA and AA were extracted from brains of treated mice using a modified Bleigh and Dyer method and measured using UHPLC-MS/MS.

Results: RBE increased CD36 and decreased Aβ1-42 protein levels in the neuroinflammatory mouse model. Interestingly, RBE increased DHA and EPA levels but not AA in our mouse model brains.

Conclusions: This work indeed indicates a role for PPARγ in the cognitive enhancement mechanism of RBE and that brain PUFAs are affected by RBE treatment.
THE ROLE OF ESTRADIOL-DEPENDENT MITOCHONDRIAL BIOGENESIS IN THE COGNITIVE ENHANCEMENT EFFECTS OF METHYLENE BLUE

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Aims: Methylene Blue (MB) is currently under investigation as a potential treatment for Alzheimer’s disease. However, its mechanism of action is currently under examination. MB has been previously shown to alter Amyloid-beta(Aβ) aggregation and improve mitochondrial functions. Aβ has been shown to interact with the mitochondrial enzyme amyloid-beta-binding-alcohol-dehydrogenase (ABAD), which is responsible for the estrone/estradiol conversion. The Aβ-ABAD interaction was previously implicated in mitochondrial dysfunction. Preventing the Aβ-ABAD interaction preserves the normal functioning of ABAD, protecting mitochondrial functions and preventing Aβ-induced toxicity. Previously, we reported that MB influences Aβ-ABAD complex interaction in a neuroinflammatory mouse model, increasing estradiol levels. The current project aimed to test whether the increase in estradiol levels is related to enhanced cognition. Furthermore, due to the effects of MB on the mitochondria and the findings that estradiol is able to enhance mitochondrial biogenesis pathway, we further aimed in testing whether this pathway is activated by MB.

Methods: An LPS induced neuroinflammatory mouse model was used in this study. Mice were treated with MB or pre-treated with estrogen receptor blocker (ICI182,780) before MB. The novel object recognition and the Y-maze tests were performed for assessing their memory. Mice were then sacrificed and their brains were used for gene expression assays and H&E staining.

Results: Treatment with MB enhanced cognition, elevated PGC1-alpha mRNA levels, and decreased the appearance of neurodegeneration. Interestingly, pre-treatment with ICI182,780 reversed the effects of MB on cognition, PGC1-alpha levels, and neurodegeneration.

Conclusions: These results suggest that MB may exert its neuroprotective effects through an estrogen-dependent mitochondrial biogenesis pathway.
Bisdemethylcurcumin Loaded Ferritin Nanoparticles as a Therapeutic Agent Against Alzheimer’s Disease


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Aims: Bisdemethylcurcumin (BDC) might be useful inflammation inhibitor in AD patients. BDC is almost insoluble in water, poorly absorbed by the organism, and rapidly degraded. To solve this issue, we developed a nanoformulation of BDC based on H-Ferritin nanocages (HFn).

Methods: We tested the solubility and stability of the BDC-HFn and its ability to transverse the blood-brain barrier (BBB). We tested the effect BDC-HFn on PBMCs from AD and controls to evaluate the transcriptomic profile by RNA-seq.

Results: By using HFn as a carrier, it was possible to develop a nanoformulation with a mean diameter of 11 nm to drastically improve the solubility of BDC and extend the stability for more than 24 hours. The BDC-HFn can bind endothelial cells from the cerebral cortex and cross through a BBB in vitro model. Transcriptomic analysis showed that a total of 630 Differentially Expressed RNAs (DEG), 350 up and 280 down-regulated between AD and controls untreated. The comparison between AD patients before and after BDC-HFn treatment showed the major number of DEG (2517). Pathway analysis showed that chemokine and macrophage activation are different between AD patients and controls and after BDC-HFn treatment.

Conclusions: Our data showed how Hfn-BDC could improve the pharmacokinetic properties of the drug and that significant differences are present in the gene expression between the same patients before and after Hfn-BDC treatment, particularly in genes associated with inflammation. Moreover, inflammatory genes those are up-regulated between AD and CTR, after BDC-HFn treatment converted in down-regulated suggesting a possible therapeutic approach.
TREADMILL EXERCISE IMPROVES COGNITIVE IMPAIRMENT BY ATTENUATINGAMYLOID-B AND TAU HYPERPHOSPHORYLATION LEVELS VIA BRAIN IRONDYSHOMEOSTASIS IN TRANSGENIC MOUSE MODEL OF ALZHEIMER'S DISEASE

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Aims: Alzheimer's disease (AD) is known to be a major lesion in the accumulation of neurofibrillary tangles and β-amyloid due to hyperphosphorylation of Tau protein, and its association with abnormal brain iron metabolism is increasing recently. Therefore, the purpose of this study was to observe the effect of treadmill exercise on the regulation of cerebral iron homeostasis and β-amyloid and Tau hyperphosphorylation in Alzheimer's disease.

Methods: Twenty mice with transgenic mice and ten mice with non-transgenic were divided into three groups: non-tg-control(CON, n=10), tg-control(AD-C, n=10), and tg-exercise(AD-TE, n=10). AD-TE group was performed to progressive TE for 12 weeks. And then, we measured the cognitive function using water maze test and passive avoidance task and, brain cortex were evaluated to determine whether any changes in the oligomer Aβ, tau, apoptotic-related factors biogenesis.

Results: The treadmill exercise maintained iron homeostasis by reducing the accumulation of AD-related abnormal iron in the hippocampus of the brain through aging APP-C105 mice. Next, we investigated whether treadmill exercise affects β-amyloid accumulation and Tau hyperphosphorylation according to iron homeostasis regulation. We confirmed β-amyloid accumulation and Tau hyperphosphorylation due to abnormal iron accumulation in Alzheimer's disease, which also resulted in neuronal cell death and cognitive decline. However, it was confirmed that treadmill exercise attenuated β-amyloid accumulation and Tau hyperphosphorylation, and improved neuronal cell death and cognitive function.

Conclusions: These results suggest that treadmill exercise modulates iron homeostasis to alleviate neuronal cell death and cognitive decline. Therefore, treadmill exercise and therapeutic strategies to maintain iron homeostasis may be useful in AD patients.
A NEW ROUTE OF DRUG DELIVERY FOR NEURODEGENERATIVE DISEASES USING A FULLY IMPLANTABLE DEVICE: PROOF OF CONCEPT IN MICE

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Aims: Crossing the BBB is one of the main challenges of anti-amyloid drugs. Many drug delivery techniques are under development with the aim of facilitating BBB crossing. We propose a radically new way of administering drugs for proteinopathies using a fully implantable device with access to the CSF and a subcutaneous reservoir. Here we present the proof of concept in rodents.

Methods: A miniaturized prototype of the device was designed and manufactured. AD and wild type mice were implanted (intraventricular catheter, subcutaneous reservoir) for up to 8 weeks and randomized to receive anti-amyloid mAbs or vehicle. Feasibility, safety and efficacy studies were carried out.

Results: The device was successfully implanted in all mice. Dosing of drugs/vehicle was performed easily via percutaneous route. The therapy showed to be feasible and safe. Preliminary efficacy studies were positive.

Conclusions: This system provides a new route of delivery of drugs for neurodegenerative diseases. It offers a number of key advantages in terms of efficacy and safety over peripherally administered drugs.
UPDATE PHASE 2 STUDY OF ABVAC40, AN ACTIVE VACCINE ANTI-AB40 IN PATIENTS WITH MILD COGNITIVE IMPAIRMENT OR VERY-MILD ALZHEIMER’S DISEASE

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Aims: One of the main hallmarks of Alzheimer disease (AD) is the deposition of amyloid Aβ peptide in the neuropil and blood vessels. Immunotherapy against Aβ peptide is the most promising approach to slow the progression of AD. Araclon-Biotech is developing an active vaccine against Aβ40 peptide, one of the most abundant in senile plaques and the dominant in vascular deposits, that has been associated with earlier onset of dementia.

Methods: In 2017 Araclon-Biotech initiated a Phase 2, 24-month, multicenter, randomized, double-blind, placebo-controlled trial in patients with amnestic mild cognitive impairment (a-MCI) or very mild AD (Vm-AD) to investigate the safety, tolerability, and immune response of ABvac40 (Part A) (NCT03461276). In July-2020, a protocol amendment was approved whereby the blind phase (Part A) was shortened from 24 to 18-months and an additional 18-months cross-over study (Part B) was added.

Results: 124 patients aged 55 to 80 have been included (a-MCI, n= 80; Vm-AD, n= 44). Baseline demographics and characteristics were balanced. A total of 101 patients have completed Part A and 77 of which have finally transitioned to Part B. Two blind interim analysis was done; in July-2019 (30 patients completed 6- months) and in December-2020 (124 patients completed 6-month). Notably, both analyses concluded that there were no safety and efficacy concerns to jeopardize the continuity of the study.

Conclusions: Part A was completed as previously planned and Part B is progressing as scheduled. Safety profile of ABvac40 and cross-over design will be presented in the congress. Top line results are expected for Q1-2022.
BRAIN DELIVERY OF PARKIN WITH CELL-PERMEABILITY REDUCES AGGREGATED B-AMYLOID AND RECOVERS COGNITIVE FUNCTION IN FIBRIL B-AMYLOID-INDUCED ANIMALS

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Aims: Alzheimer's disease (AD) is a neurodegenerative disorder, which is pathologically characterized by β-amyloid (Aβ) plaques and neurofibrillary tangles in specific regions (e.g., hippocampus and cortex) of the brain. Impairment of mitochondrial function by these abnormally aggregated proteins is a fundamental phenomenon in AD. Previously, an improved cell-permeable Parkin protein (iCP-Parkin) containing an advanced macromolecule transduction domain (aMTD), a hydrophobic cell-penetrating peptide, had been developed as an anti-neurodegenerative therapeutic agent, capable of intraneuronal delivery across the blood-brain barrier (BBB) and neuroprotection by the replacement of damaged mitochondria. In particular, the current study shows that iCP-Parkin ameliorates AD-induced neurodegenerative symptoms by recovering mitochondrial dysfunction caused by aggregated Aβ.

Methods: AD mouse model was induced by injecting fibril amyloid-beta (fAβ) into the brain through stereotaxic surgery.

Results: As the result in Aβ-treated HT22 cells, relative oxidative stress levels are reduced by 103% at 3 hours with iCP-Parkin treatment. Next, the treatment of iCP-Parkin (25, 50 mg/kg, 3 times/week for 4 weeks) shows a significant recovery of cognitive function, as assessed by Y-maze tests (25 mg/kg: 89%; 50 mg/kg: 105%) in fibril Aβ1-42-induced AD mice. The level of Aβ plaques is also suppressed (~70%), as measured by dot-blot analysis. Lastly, 5.6% (brain/total tissue distribution ratio) of injected iCP-Parkin is shown to be delivered to the brain based on LC-MS/MS analysis.

Conclusions: In conclusion, the present outcome evidently supports the ability of iCP-Parkin to remove Aβ plaques and recover from cognitive dysfunction with mitochondria recovery, demonstrating its potential as a novel therapeutic agent against AD.
CONTRIBUTIONS OF MICROGLIA AND APOE TO AMYLOID-FACILITATED TAU-PATHOLOGY AND DOWNSTREAM NEURODEGENERATION IN AN A/T/N PRECLINICAL MODEL


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**Aims:** Alzheimer’s disease is characterized by the presence of amyloid plaques (A), neurofibrillary tangles (T) and neurodegeneration (N), constituting ATN pathology. We here studied the role of microglia and APOE on progressive development of ATN pathologies. Furthermore, a role of ApoE in progression along the ATN axis is increasingly emerging, further supported by the identification of a protective ApoEch/ch mutation in an EOAD- mutation carrier.

**Methods:** We used a model displaying strong amyloid facilitated bilateral tau propagation following tau-seeding, associated with hippocampal and cortical atrophy, thereby recapitulating robust ATN pathology. We now combined this model with single cell sequencing, microglial elimination and crossings with ApoE-/- mice, to gain insight in the role of ApoE and microglia in ATN pathology development.

**Results:** Single-cell RNA sequencing revealed that ATN pathology exacerbated microglial activation towards disease-associated microglia (DAM) states, with a significant upregulation of Apoe as compared to amyloid-only models. This further emphasizes a potential role of ApoE in progressive ATN pathologies currently under investigation in our lab. Importantly, Colony-Stimulating Factor 1 Receptor inhibition demonstrated a contributory role of microglia in amyloid facilitated tau pathology and downstream neurodegeneration. CSF1R inhibition, resulted in a preferential depletion of non-plaque-associated microglia thereby significantly attenuating tau pathology and neuronal atrophy downstream of amyloid pathology, indicating their detrimental role during ATN progression. We currently assess the role of ApoE in progressive ATN pathologies.

**Conclusions:** Together, our data reveal a role of microglia and potentially ApoE to amyloid facilitated tau pathology and neurodegeneration, in a preclinical model displaying ATN pathology.
POSTGRADUATE OPEN-LABEL ROLLOVER STUDY: EVALUATION OF SUBCUTANEOUS GANTENERUMAB LONG-TERM SAFETY, TOLERABILITY, AND EFFICACY IN PARTICIPANTS WITH ALZHEIMER’S DISEASE

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Aims: Gantenerumab, a fully human anti-amyloid beta (Aβ) monoclonal antibody in development for Alzheimer’s disease (AD), binds to and removes Aβ species. Two ongoing multi-centre, randomized, double-blind, placebo-controlled, Phase III studies, GRADUATE I (NCT03444870) and GRADUATE II (NCT03443973), assess the efficacy and safety of subcutaneous gantenerumab (1,020 mg monthly dosage) in early (prodromal-to-mild) AD. Here, we describe the study design of the open-label, multicenter, rollover study (PostGraduate, NCT04374253), enabling the evaluation of gantenerumab’s long-term safety, tolerability, and efficacy in participants from GRADUATE I and II.

Methods: Participants treated with gantenerumab who completed either parent study (up to 2,032 participants) will receive subcutaneous gantenerumab (510 mg every 2 weeks [Q2W]) for 2 years. Participants naive to gantenerumab will up-titrate in 3 steps (120 mg, 225 mg, 510 mg every 4 weeks) to target 510 mg Q2W. Participants will remain blinded to previous treatment assignment.

Results: The primary objective is to evaluate safety and tolerability by assessing adverse events, physical examinations, vital signs, laboratory tests, suicidality, amyloid-related imaging abnormalities, and injection-site reactions. Secondary and exploratory objectives include evaluation of efficacy (measures of cognition, function, quality of life, and caregiver burden aligned with parent studies), pharmacokinetics, anti-drug antibodies, and longitudinal biomarkers (amyloid and tau positron emission tomography in respective substudies, fluid biomarkers, magnetic resonance imaging).

Conclusions: PostGraduate is a scientifically valuable study of safety, clinical measures, and fluid and imaging biomarkers in a large group of participants, enriched for amyloid positivity and short-term memory loss, and treated continuously for up to 4 years.
ADUCANUMAB PHASE 3 STUDIES: EXPOSURE-RESPONSE ANALYSIS EVALUATING THE RELATIONSHIP BETWEEN AMYLOID REMOVAL AND SLOWING OF CLINICAL DECLINE ON CDR-SB SCORES

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Aims: Determine the relationship between amyloid beta plaque removal and slowing of clinical decline in the EMERGE (NCT02484547) and ENGAGE (NCT02477800) Phase 3 studies of aducanumab using a model-based approach.

Methods: Exposure-response modeling evaluating the relationship between amyloid removal as measured by standardized uptake value ratio (SUVR) and changes in clinical dementia rating sum of boxes (CDR-SB model). SUVR was measured in 949 of 3282 subjects at Baseline, Weeks 26 and 78. For subjects without SUVR observations, missing observations were imputed using predictions adjusted for baseline covariates included in the exposure-SUVR model. The final dataset included 3282 patients and 11088 CDR-SB observations, collected at Baseline, Weeks 26, 54 and 78. Aβ removal and its impact on clinical outcomes between the studies were investigated.

Results: To account for heterogeneity, a mixture model was assumed to identify 3 classes of disease progression (i.e., slow, typical, and fast progressors). The exposure-SUVR-CDR-SB model incorporated therapeutic activity of aducanumab via fractional changes in SUVR from baseline as an additive effect on the disease progression rate. Incorporating SUVR in the exposure-SUVR-CDR-SB model resulted in an improved fit explaining more of the variation in observed clinical effect in CDR-SB compared to a simpler model. Further, no detectable difference in aducanumab effect as measured by SUVR on CDR-SB was observed across EMERGE and ENGAGE.

Conclusions: A robust model demonstrated a relationship between SUVR and CDR-SB and provides evidence of consistent aducanumab pharmacology across both Phase 3 studies.
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Aims: Small conductance calcium-activated potassium (SK) channels have been implicated in neurological diseases, including Parkinson's and Alzheimer's disease. Recently, we developed novel pharmacological SK2 channel openers, based on compound affinity to the SK2 channel binding pocket. We have screened 11 potential SK2 channel positive modulators and selected 2 compounds for its neuroprotective role in ferroptosis models and compare its potency to classic SK channel activator, CyPPA.

Methods: Microelectrode array was used to show the impact of 2 compounds on firing rates in murine primary neurons. MTT assays, xCELLigence measurements and PI were combined to determine cell viability of neuronal HT22 cells. Additionally, we examined the effect them on RSL3-induced toxicity, mitochondrial ROS production and mitochondrial calcium uptake via FACS measurements.

Results: We determined the IC₅₀ of several novel potential activators of SK channels in neuroprotective studies against ferroptosis and established 2 compounds as potent neuroprotective compounds and SK channel openers. Both screened compounds reduced the neuronal firing rates induced by glutamate in primary neuronal cells. It prevented ferroptosis in a concentration-dependent manner, as detected by MTT assay and xCELLigence measurements. PI FACS analysis showed that the increased number of positive stained cells after RSL3 exposure were largely attenuated by compounds. Both compounds prevented the mitochondrial calcium uptake, mitochondrial ROS production mediated by RSL3 challenge. One of them effort neuroprotection at nanomolar.

Conclusions: These data demonstrate a novel class of compound that open SK channels mediate neuroprotection in conditions of oxidative stress at nanomolar, being a breakthrough and potential therapeutic target.
A PHASE 3 CLINICAL TRIAL PROTOCOL TO EVALUATE THE EFFICACY AND SAFETY OF NA-831 IN SUBJECTS WITH EARLY ALZHEIMER'S DISEASE

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Aims: This phase 3 study consists of a Core and Open Label Extension (OLE) Phase in 465 participants with Early Alzheimer's Disease (EAD), and will be conducted to evaluate the efficacy and safety of NA-831. The Core is a 52-week treatment, multicenters, double blind, placebo controlled parallel group study.

Methods: Core Study: Participants will receive one capsule of 30 milligram (mg) NA-831 orally once a day in the morning. The core study will be double blinded. Placebo Comparator: The core study will be double blinded. Experimental: Open Label Extension Phase: Participants completing the core study will receive one 30 milligram (mg) NA-31 capsule orally once a day in the morning.

Results: Key Outcome Measures: 1. Core Study: Change From Baseline in the Clinical Dementia Rating - Sum of Boxes (CDR-SB) Score at 48 Weeks [ Time Frame: Baseline, Week 52 ] 2. Open-Label Extension Phase: Number of Participants With Treatment-Emergent Adverse Events (AEs) [ Time Frame: Up to Week 52 of Extension Phase ] Secondary Outcome Measures: Cognition-13 (ADAS-Cog-13) at Weeks 24, 52 [ Time Frame: Baseline, Week 24, Week 52 of Extension Phase ] CORE STUDY: Mild cognitive impairment due to AD or mild AD dementia including 1. MMSE score equal to or greater than 24 2. CDR global score of 0.5 3. CDR Memory Box score of 0.5 or greater

Conclusions: The Phase 3 clinical trial will be conducted in 25 sites in the US and several countries. The details of the Phase 3 methodology and protocol will be presented and discussed.
POSTERS

BENEFICIAL EFFECTS OF MASUPIRDINE (SEROTONIN RECEPTOR SUB-TYPE 6 ANTAGONIST) ON PSYCHOSIS IN PATIENTS WITH ALZHEIMER’S DISEASE

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Aims: Aim of the present investigation was to evaluate the effect of masupirdine on psychosis symptoms associated with Alzheimer’s disease (AD) using the delusions and/or hallucinations (psychosis) domains of the 12-item neuropsychiatric inventory (NPI-12) assessment scale.

Methods: Masupirdine was studied in a multicenter, randomized, double-blind, parallel group, 26-week, placebo-controlled proof of concept phase-2 clinical trial in subjects with moderate AD (NCT02580305). Subgroup analyses were carried out on the psychosis domains of the NPI-12 scale. Analyses were based on the independent patient subgroups with baseline symptoms and/or symptoms emergence. A mixed-effects model for repeated measures was used to determine the effect of masupirdine on psychosis symptoms in modified intention to treat population.

Results: In the subgroup of population with baseline psychotic symptoms and/or symptoms emergence, a significant reduction (p<0.05) in psychosis score was observed in the masupirdine 50 mg treatment arm at Week 4 & 13. A trend was observed in the masupirdine 100 mg (p<0.1) treatment arm at Week 26 as compared to placebo treated arm. Effect size (Cohen’s d) observed with masupirdine treatment (50 & 100 mg) was 0.32 - 0.50 and 0.25 - 0.39 at the end of 13 and 26 weeks, respectively. Masupirdine also showed beneficial effects on cognition in patients with psychosis.

Conclusions: Further exploration is warranted to confirm the beneficial effects of masupirdine on psychosis associated with AD.
NOVEL DUALLY-ACTING ACETYLCHOLINESTERASE INHIBITORS WITH N-METHYL-D-ASPARTATE RECEPTOR ANTAGONISM AS POTENTIAL SYMPTOMATIC DRUGS AGAINST ALZHEIMER'S DISEASE

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Aims: The aim of this study was to investigate the effect of NMDAR antagonists on the glutamatergic system and the potential benefits of dually acting compounds (targeting both the ChE and NMDAR) on cognition and possible protection from neurodegeneration associated with Alzheimer's disease (AD). The ultimate goal is to develop novel clinical candidates for the palliative treatment of AD.

Methods: Our hypothesis was that memantine-associated alteration in the total protein expressions of the excitatory synapse complex and NMDARs counteracts the neurodegeneration-associated cognitive decline observed in AD patients. The hypothesis is based on the fact that i) memantine is currently available on the market for the treatment of AD patients and that ii) acetylcholinesterase inhibitors, specifically tacrine, exhibited beneficial effect in AD patients probably due to a complex action involving the interaction with the NMDARs.

Results: We have developed a series of 30 novel tacrine derivatives whose blocking efficacy differs among different compounds and receptors using electrophysiology with HEK293 cells expressing the defined types of NMDARs. Selected compounds potently inhibited both GluN1/GluN2A and GluN1/GluN2B receptors. Using in vivo experiments in rats we observed that, unlike MK-801, the tested novel compounds did not induce hyperlocomotion in open field, and also did not impair prepulse inhibition of startle response, suggesting minimal induction of psychotomimetic side effects.

Conclusions: We conclude that tacrine derivatives are promising compounds since they are centrally available subtype-specific inhibitors of the NMDARs without detrimental behavioral side-effects. Study was supported by Czech Science Foundation project. No. 20-12047S and by project ERDF IT4N no. CZ.02.1.01/0.0/0.0/18_069/0010054.
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Aims: STRiatal Enriched protein tyrosine Phosphatase (STEP61) enzyme has its importance in neural circuits extending functional pathways through different areas of brain involved in motor reflexes, cognition, learning and memory. Activity of STEP61 increases abruptly in AD, which causes undesired Protein Protein Interactions(PPIs) leading to dementia. Inhibition of STEP61 involving PPIs can be a great area of therapeutic interventions for treatment of Alzheimer’s dementia.

Methods: In this study, We have used in-silico computer aided drug designing and computational biology methods for mapping the active conformation of binding site of STEP61. We further designed and screened a focused library of peptidomimetics (38000 compounds) and phytoconstituents (6670 compounds) with desirable calculated/predicted physiochemical properties specifically for inhibitory activity against STEP61. A pharmacophore was also generated using potential hits. Glide score was used for binding affinity prediction and ligand ranking. The dynamic simulation was performed for 2ns and analyzed using root mean square deviation(RMSD). Schrödinger Drug Discovery Suite Release 2019 and MOE software were used for performing various molecular modeling operations and dynamics respectively.

Results:

<table>
<thead>
<tr>
<th>Residue No.</th>
<th>Aminoacid</th>
<th>Nature</th>
</tr>
</thead>
<tbody>
<tr>
<td>435</td>
<td>Tryptophan</td>
<td>Non-polar, neutral</td>
</tr>
<tr>
<td>436</td>
<td>Aspartic acid</td>
<td>Polar acidic</td>
</tr>
<tr>
<td>437</td>
<td>Proline</td>
<td>Non-polar, neutral</td>
</tr>
<tr>
<td>470</td>
<td>Valine</td>
<td>Non-polar hydrophobic</td>
</tr>
<tr>
<td>471</td>
<td>Histidine</td>
<td>Basic (+ve) H bond donor(NH)</td>
</tr>
<tr>
<td>472</td>
<td>Cysteine</td>
<td>Polar uncharged</td>
</tr>
<tr>
<td>473</td>
<td>Serine</td>
<td>Polar uncharged</td>
</tr>
<tr>
<td>474</td>
<td>Alanine</td>
<td>Non-polar hydrophobic</td>
</tr>
<tr>
<td>475</td>
<td>Glycine</td>
<td>Polar uncharged</td>
</tr>
<tr>
<td>476</td>
<td>Lysine</td>
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<tr>
<td>477</td>
<td>Glycine</td>
<td>Polar uncharged</td>
</tr>
<tr>
<td>478</td>
<td>Arginine</td>
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<td>479</td>
<td>Threonine</td>
<td>Polar uncharged</td>
</tr>
<tr>
<td>480</td>
<td>Glycyl</td>
<td>Polar uncharged</td>
</tr>
<tr>
<td>481</td>
<td>Cysteine</td>
<td>Polar uncharged</td>
</tr>
</tbody>
</table>

The top 10 potential hits identified by extra precision molecular docking studies showed great binding affinity/docking score (-10 to -14 range) and the dynamics studies reflected consistent stable interactions throughout the MD simulation with low potential energy and RMSD of <2.

Conclusions: The top hits are promising molecules with potential for further development as inhibitors of STEP61 in the treatment of AD associated dementia. The hits were synthesized and will be considered for in vitro and in vivo analysis.
Aims: A Ph1b clinical trial was conducted (SNAP, NCT03522129) to verify target engagement of the S2R modulator CT1812 in Alzheimer’s disease (AD) patients by measuring changes in Aβ oligomers (AβOs) in CSF, as previously demonstrated in AD transgenic mice.

Methods: A randomized, double-blind, placebo-controlled trial of CT1812 was conducted in mild to moderate AD patients. CSF draws from a lumbar epidural catheter were taken hourly over 28 hours, at baseline and after a single oral dose of CT1812 (560 mg, two patients) or placebo (one patient). CSF AβO levels were measured via microimmuno electrode and by native western blots with oligomeric Aβ selective antibodies. Aβ40/42 monomer levels were measured via ELISA; and CSF CT1812 concentrations were determined via LC/MSMS.

Results: A robust increase (~2.5-5 fold) over time in CSF AβO concentrations was measured by microimmuno electrode in both patients given CT1812, whereas no increase in AβO was observed in the placebo-treated patient. This finding was observed using two independent methods which showed a high degree (r=0.74) of inter-assay congruence. The increase in AβO levels was specific to the oligomeric form, as monomeric levels were not altered in a treatment-dependent manner and increased modestly over time in all three patients. These data are consistent with the mechanism of action of CT1812 and its selective action on AβO elaborated in preclinical studies.

Conclusions: Results demonstrate the first clinical evidence of target engagement of CT1812 and support that CT1812 can engage S2R in brain and selectively mobilize toxic AβOs from their receptors in AD patient brains.
PROTEOMIC ANALYSIS OF CSF IN A PHASE 2 CLINICAL TRIAL IN ALZHEIMER’S PATIENTS TO IDENTIFY PHARMACODYNAMIC BIOMARKERS OF THE S2R MODULATOR CT1812

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Aims: Unbiased quantification of CSF proteomes from Alzheimer’s Disease (AD) patients given the small molecule S2R modulator CT1812 or placebo, to identify pharmacodynamic (PD) biomarkers of target/pathway engagement and disease modification.

Methods: Tandem-mass tag mass spectrometry (TMT-MS) was performed on baseline and end of study CSF samples taken from the first 24 participants that completed the Ph2 randomized, double-blind, placebo-controlled trial (SHINE; NCT03507790), to test two doses of CT1812 given once daily for 6 months in mild to moderate AD patients. CSF proteomes were compared to within-study pooled AD and non-demented control CSF reference standards from the Emory ADRC to compare protein levels in the SHINE-A cohort with well-characterized AD CSF and to assess treatment effects through differential expression and pathway analyses.

Results: Across all samples, 2,182 CSF proteins were detected. Strong correlations (r>0.80) between TMT-MS and clinically validated ELISAs for canonical AD biomarkers (Tau, NFL) were observed across participants and timepoints, validating TMT-MS as a quantitative method. Differential expression analyses identified proteins altered (p<0.05) in CT1812 vs placebo CSF, and hierarchical clustering and PCA analyses demonstrated stratification of patients by treatment. Comparisons to reference standards illuminated proteins disrupted in or genetically linked to AD that were normalized by CT1812 to control levels. Pathway analysis identified statistically significant pathways altered by CT1812, including that tied to amyloid and synaptic function.

Conclusions: This study enabled the identification of candidate pathway engagement and disease modification biomarkers that can be tracked in future studies, and provides additional clinical support that CT1812 is a promising therapeutic approach to AD.
Aims: AD is a multifactorial slow and progressive dementing disease that combines several mechanisms. So far, most efforts have been focused on a single mechanism. Two pathological processes leading to the so-called amyloid deposits and neurofibrillary degeneration are the bases of AD. Our interest is to identify novel pharmacological targets, propose and develop novel drugs ideally targeting simultaneously APP metabolism and Tau pathology. We developed two series of compounds derived from either N,N'-disubstituted piperazines (series A, Melnyk et al. WO 2006 051489; Melnyk et al. ACS Chem Neurosci 2015; Sergeant et al. Neurobiol Dis 2019) or anilinoquinolines (series B, Delacourte et al. EP2010069897; Gay et al. Bioorg Med Chem 2018) that demonstrated activities on both processes and are at the origin of new families. The lead compound of series A is currently starting phase II clinical trials.

Methods: A ligand-based pharmacophore modeling approach, coupled with de novo design was implemented. In vitro experiments were performed. Well-characterized transgenic mice were used and cognition benefits were measured using behavioural tests.

Results: Several families of compounds were designed and synthesized with expected profile (Gay et al. Eur J Med Chem 2018, Tautou et al. Front Pharmacology 2021). One compound restored the short-term memory in Thy-Tau22 mice and the long-term spatial memory in APP/PS1 mice. These beneficial effects were associated with a reduced neurofibrillary degenerating process and a decreased amount of amyloid plaque load.

Conclusions: Using a ligand-based approach, original multi-action compounds were designed. Structure-activity relationship allowed the study of the mechanism of action.
SAFETY AND FEASIBILITY OF PHYTOSERM - A SELECTIVE ESTROGEN B-RECEPTOR PHYTOESTROGEN FORMULATION - FOR IMPROVING MENOPAUSAL SYMPTOMS AND COGNITIVE FUNCTION: PHASE 1B/2A RANDOMIZED CLINICAL TRIAL

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Aims: To assess safety, tolerability, pharmacokinetics and feasibility of the PhytoSERM formulation (genistein, daidzein, and S-equol) following a single dose and multiple daily doses for 12 weeks in menopausal women with cognitive complaints.

Methods: We conducted a randomized, placebo-controlled trial of 12 weeks duration comparing 50 and 100 mg of PhytoSERM with placebo for non-cognitively impaired, perimenopausal women ages 45 to 60, with intact uteri and ovaries, at least one cognitive complaint, and one vasomotor-related symptom. Primary objectives were to assess safety and tolerability of both oral doses of PhytoSERM taken daily. Secondary objectives were to evaluate potential indicators of efficacy on cognition and vasomotor symptoms over 12 weeks.

Results: Seventy-one women were randomized to treatment; 70 were evaluated at 4 weeks and 5 did not complete 12 weeks. Overall, 87% were greater than 90% compliant with their medication. There were no statistically significant effects on either vasomotor composite or the neuropsychological composite over 12 weeks (p=0.25 and p=0.57, respectively. However, change from baseline over 12-weeks in hot flash frequency alone was significantly lower in the 50mg group compared to placebo (p=0.04). Adverse events occurred in 16.7% (n=4) placebo, 39.1% (n=9) 50 mg per day, and 29.2% (n=7) 100 mg per day treated participants.

Conclusions: The PhytoSERM formulation appeared safe and well-tolerated at 50 and 100 mg daily doses. Based on safety outcomes, and vasomotor symptoms and cognitive outcomes at 12 weeks an optimal daily dose of 50mg was established for a phase 2 efficacy trial.
ACCELERATING ALZHEIMER’S DISEASE DRUG DEVELOPMENT THROUGH PRECOMPETITIVE DATA SHARING FOR THE DEVELOPMENT OF REGULATORY-ENDORSED MODEL-INFORMED DRUG DEVELOPMENT TOOLS

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Aims: There is a pressing need for an optimized quantitative basis for designing clinical trials, particularly in the early stages of Alzheimer’s disease (AD). The Critical Path for Alzheimer’s Disease (CPAD) Consortium serves as a pre-competitive, neutral convenor for generating novel and actionable regulatory-endorsed drug development tools (DDTs) for optimization of clinical trial design.

Methods: Patient-level data, from contemporary Phase II and III AD clinical trials and observational studies, are transferred through formal data contribution agreements. After data curation and standardization, integrated data are evaluated for the utility of available variables (outcomes, biomarkers, demographics, genetics, etc.). Analysis subsets are derived, incorporating individual or multivariate disease progression markers and signatures. Next, quantitative disease progression models and DDTs are generated to better understand pathology and treatment effects across the disease continuum, and to facilitate decision making for accelerating drug development in AD.

Results: Per August 2021, CPAD’s data repository contains 51 studies with 33,868 individual anonymized patient records, with a rich source of key amyloid, tau, and neurodegeneration biomarkers (biofluids and imaging). Base models are being developed using parametric mixed-effects approaches with selection based on goodness-of-fit plots, for the FDA-proposed stages of the AD continuum. Covariate analyses will identify variables that constitute relevant predictors of baseline severity and disease progression rates. Model-based clinical trial simulation tools will be submitted for endorsement by regulatory agencies.

Conclusions: The precompetitive data acquisition and analysis pioneered by CPAD is fundamental to the collaborative generation of actionable quantitative DDTs for accelerating and advancing AD drug development.
Aims: The extent to which newly developed blood-based biomarkers of Alzheimer's disease could improve screening for secondary prevention trials is unclear.

Methods: Plasma Aβ42/Aβ40, P-tau217, NfL, and GFAP levels, along with amyloid PET and cognition (PACC), were measured at baseline in 181 cognitively unimpaired (CU) participants (mean [SD] age of 72.9 [5.3] years; 61.9% female; mean [SD] education of 11.9 [3.4] years) from the Swedish BioFINDER-1 study. We identified a parsimonious set of biomarkers to model amyloid PET status, then tested whether the resulting model reduced cost to recruit CU participants into a theoretical clinical trial requiring abnormal amyloid PET levels for enrolment. A cost-benefit analysis was performed.

Results: A parsimonious model of plasma Aβ42/Aβ40, P-tau217, and GFAP significantly reduced the cost of recruiting CU individuals with abnormal amyloid PET when the PET was assumed to cost 8 times more than plasma biomarkers (cost reduction = -37.5% [-49.2, -25.7] for a plasma cutoff of 75% probability of being amyloid positive; cost reduction = -31.2% [-24.3, -38.2] using a cutoff of 50% probability; cost reduction = -9.9% [-15.6, -4.2], using a cutoff of 50% probability). Theoretical cost savings were maximized at 38.1% ([31.2, 44.9], P < 0.001) with a 16x PET:plasma cost ratio and a risk threshold of 0.25. At the same risk threshold, plasma biomarkers also provided a 22.3% increase in expected amyloid PET-positive rate over using demographics and PACC alone (P < 0.0001).

Conclusions: As a pre-screening tool, plasma biomarkers could significantly lower amyloid PET-related screening costs in secondary prevention trials of AD.
Aims: Objective: Evaluate the safety and efficacy of NeuroEPO in the treatment of patients with mild-to-moderate Alzheimer’s clinical syndrome.

Methods: A double-blind, randomized, placebo-controlled trial enrolled 174 subjects with mild-to-moderate Alzheimer’s clinical syndrome, was conducted. Patients were randomized to NeuroEPO 0.5 mg or 1.0 mg or placebo, and treated 3 times/week for 48 weeks. Primary endpoint was the change from baseline in the 11-item AD Assessment Scale-Cognitive subscale (ADAS-Cog11). Secondary endpoints included CIBIC+, GDS, MoCA, NPI, neuropsychological battery, EGG and cerebral perfusion measured by SPECT.

Results: Surprisingly NeuroEPO treatment reduced ADAS-Cog11 after 48 weeks in -4.0 ± 4.0 and -5.0 ± 5.0 units respectively for the groups of 0.5 and 1.0 mg dose, while placebo group increased ADAS-Cog11, as expected, in 4.0 ± 6.0. NeuroEPO treatment also induced a statistically significant improvement in GDS, MoCA, NPI and neuropsychological battery as compared to placebo. About electroencephalography, 72% of the NeuroEPO-treated patients stabilized or decreased the values of EEG (P=.003) vs. placebo group. Reference to cerebral perfusion, 56% of the NeuroEPO-treated patients improved their parieto-temporal perfusion, whereas none of placebo group improvement. No serious adverse events related with neuroEPO were reported.

Conclusions: Overall NeuroEPO significantly improved clinical outcomes with a good safety profile in patients with mild-to-moderate Alzheimer’s clinical syndrome.
AI-DRIVEN PHYSICIAN REFERRAL NETWORK RECRUITMENT INITIATIVE TO DRIVE VOLUME, SPEED AND EFFICIENCY FOR CLARITY AD, A PHASE 3 TRIAL IN EARLY AD

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Aims: Recruitment to Alzheimer’s disease (AD) trials is a critical challenge in clinical development. The objective of the SiteRx platform is to use electronic health records (EHR) to identify trial participants for increasing physician engagement and clinical referrals.

Methods: SiteRx has a nationwide network of community physicians and an AI-driven process for analyzing structured and unstructured EHR data against trial inclusion/exclusion criteria, which is validated by a human reviewer. The physician introduces the trial opportunity to the trial match and initiates the referral if the patient is interested. Support services are provided to referring physicians, patients and sites to enhance patient centricity and reduce site burden. Key performance indicators included speed and conversion from referral to screening to randomization.

Results: SiteRx provided recruitment services on a late stage early AD trial during the last 3 months of enrollment. A total of 200 patients were referred, of which 90% were contacted by the site, 69% prescreened, 64% screened, and 15-38% randomized (range due to key protocol changes). Screen fail rates were comparable to study average. Eighty-one percent of referrals signed informed consent within 30 days and patients randomized 53 days from referral on average. Over 10% of referrals were Latinx. Results were achieved despite COVID-19 challenges.

Conclusions: Platforms that allow physicians to seamlessly identify and refer patients to clinical trials can improve recruitment. Bridging clinical care and clinical research has the potential to improve protocol design, site selection, enrollment timelines and diversity/inclusion in clinical trial research across various therapeutic areas.
BIOMARKER-DRIVEN INDEPENDENT CLINICAL STUDY DESIGN ON GV-971 IN CHINA AGING AND NEURODEGENERATIVE DISEASE INITIATIVE (CANDI) COHORT

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Aims: Objectives: Assess the clinical efficacy and biomarkers transformation by treating with GV-971 over 36 weeks.

Methods: This is a phase IV randomized, controlled (RCT), double-blinded, CANDI based signal-center clinical trial (Trials registration: chictr.org.cn; ChiCTR2100047830). Enrolling participants who confirmed AD with 2018 NIA-AA research framework by biomarkers supporting AD pathology (amyloid-PET, CSF related AD biomarkers, ¹⁸F-FDG-PET, and resting state functional MRI [R-fMRI]) and baseline MMSE score 12 - 26. A total of 60 subjects ages 50 to 79 will be randomized 1:1 to receive GV-971 or placebo treatment lasted 36 weeks, during which therapy is self-administered at home with the help of caregiver. Symptomatic changes will assess every 12 weeks via CDR-SB, ADAS-Cog12, MMSE, ADCS-ADL. Plasma biomarkers, peripheral blood immunity and metabolites, fecal microbiota will be assessed at baseline and after 12, 24 and 36 weeks of treatment. R-fMRI, dual mode fundus camera retinal imaging and FDG-PET will be assessed at baseline and 36 weeks.

Results: A total of 60 from CANDI cohort subjects will be enrolled and completed the full 36-week treatment period. Results of efficacy and biomarkers transformation assessment will be presented.

Conclusions: This study will be the first exploratory clinical trial to evaluate changes of brain volumn, retinal, plasma and fecal microbiota biomarkers in subjects via GV-971 treatment. Importantly, one of the goals of this trail is to analize the correlation of efficacy and changes of biomarkers by GV-971. It will be important to explore the mechanism of GV-971 in AD treatment and the pathogenesis of AD in the further.
REMOVAL OF AMYLOID BETA AGGREGATES USING TARGETED SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES

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Aims: Multiple Alzheimer’s disease (AD) clinical trials target pathogenic amyloid-β (Aβ) species using therapeutic anti-Aβ antibodies. However, recent clinical trials demonstrate immediate need for the development of new therapeutic approaches to improve efficacy and reduce passive antibody infusion side effects. The objective of this work is to develop superparamagnetic iron oxide nanoparticles (SPIONs) conjugated with anti-Aβ antibodies to improve upon existing Alzheimer’s disease therapeutics.

Methods: We combined SPIONs with Aβ antibodies to develop a therapeutic methodology for rapid and robust removal of Aβ aggregation using an external magnetic force. SPIONs conjugated with anti-Aβ antibodies selectively bind to Aβ peptides and aggregated Aβ species in our 3D human neural cell culture model of AD. We also used a vertical triculture model of AD to test the effects of alternating magnetic field on microglia Aβ phagocytosis. In vivo, we tested magnetic field-assisted antibody delivery in the brain.

Results: Application of this superparamagnetic iron oxide nanoparticles immunotherapy in our 3D human neural cell culture model of AD, followed by rapid removal of SPION-Aβ complex by an external magnet force, efficiently decreased soluble and insoluble Aβ species and accumulation of pathogenic phosphorylated tau species. Furthermore, nanoparticles and alternating magnetic field stimulation in a triculture model of AD showed increased Aβ phagocytosis by microglia cells. In 5XFAD mice, we show improved antibody delivery and a reduction in Aβ load.

Conclusions: Our results demonstrate the therapeutic potential of targeted nanotechnology in reducing both Aβ accumulation and tau pathology in vitro and in vivo.
A HYDROPHOBIC CELL-PENETRATING PEPTIDE MEDIATES BRAIN DELIVERY OF IMPERMEABLE THERAPEUTIC MOLECULES ACROSS THE BLOOD-BRAIN BARRIER VIA MODE OF DIRECT PENETRATION

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Aims: The blood-brain barrier (BBB) is a tightly-regulated & cell-based barrier that is crucial for the physiological communication and physical protection in the central nervous system (CNS). However, the development of macromolecule-based drugs against CNS diseases such as Alzheimer's disease (AD), in spite of high therapeutic potential, has been challenged due to its low BBB-penetrating capability. Here, a recombinant protein, Parkin (as a cytoprotective protein) fused with advanced macromolecule transduction domain (aMTD) has been developed to improve the BBB-permeability capable of being delivered into brain, especially undergoing CNS pathology.

Methods: Cell permeability, ATP-Glo assay and liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis have been performed in cells & animal models.

Results: As a result, aMTD-Parkin showed the improved intracellular delivery in neuronal cells as maintaining its biological functionality via direct penetration. aMTD-Parkin by utilizing its E3 ubiquitin ligase activity, showed neuroprotective effect analyzed in the ATP-Glo assay. Moreover, in fibril β-Amyloid-induced AD animals, LC-MS/MS analysis showed that 5.6% (brain/total tissue distribution ratio) of injected aMTD-Parkin was delivered to the brain, which was based on the total tissue distributed amount mediated by aMTD by subtracting the concentration of aMTD-Parkin in plasma from Parkin. In addition, the maximum amount of aMTD-Parkin was detected at the 1 H time point, whereas 3.7% of aMTD-Parkin was delivered into the brain at 30 min.

Conclusions: Overall, the current study can accurately evaluate BBB-permeability and neuronal delivery of aMTD-Parkin, suggesting the great applicability of aMTD for biologic therapeutics against neurodegenerative disease.
HEXAVALENT DESIGN OF MONOCLONAL ANTI-ALPHA-SYNUCLEIN ANTIBODY SYN-O2 IMPROVES BINDING STRENGTH TO SOLUBLE ALPHA-SYNUCLEIN OLIGOMERS

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**Aims:** Immunotherapy and -diagnostic targeting alpha synuclein (α-Syn) aggregation in Parkinson’s disease is a challenging but promising approach. Antibodies offer the high specificity and affinity needed to achieve a strong target binding while keeping the risk of off-target side effects low. However, classical antibodies often fail to bind small α-Syn aggregates, which are believed to be the disease’s most toxic α-Syn species.

**Methods:** In this study, we have created a hexavalent antibody – Hexa-Syn-O2 - derived from the α-Syn oligomer-specific antibody Syn-O2. The hexavalency was achieved through recombinant fusion of single chain variable fragments of Syn-O2 to the antibodies original N-termini. The purified antibodies’ binding kinetics were analyzed by ELISA and LigandTracer assays.

**Results:** Our analysis of binding kinetics show that Hexa-Syn-O2 binds twenty times stronger to α-Syn oligomers and protofibrils compared to SynO2 while its binding to α-Syn monomers remains negligibly weak.

**Conclusions:** This study indicates great potential of the hexavalent antibody Hexa-Syn-O2 for immunotherapeutic and -diagnostic applications as targeting small α-Syn aggregates would allow for earlier and more effective intervention in the disease’s progression.
POSTERS

AUTOMATIC SEGMENTATION OF THE PARENCHYMA VIA DEEP LEARNING

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¹IXICO plc, R&d, London, United Kingdom, ²Imperial College London, Computer Science, London, United Kingdom

Aims: The volume of brain tissue is used as a biomarker to monitor Alzheimer’s disease (AD) progression. Therefore, accurate segmentations – used to calculate volume – are highly clinically relevant. Deep learning can perform at state of the art for many computer vision problems; we present a fully automated approach that accurately delineates the parenchyma.

Methods: We present a 3D convolutional neural network (CNN), which can segment the whole brain on 3D T1 weighted images. We compare our results against ground truth and contrast performance with an industry standard atlas-based methodology (LEAP). To optimise the parameters of the CNN model a gradient-descent algorithm is employed. Random 3D patches of example manual segmentations are repeatedly fed into the architecture from a Huntington’s disease (HD) population of 320 subjects, each with 3 scans. The resulting model’s receptive field is then changed to perform inference on a whole scan. The method is then validated upon 3 AD populations (including test-retest scans), a further HD population and young and old healthynormal controls.

Results: The dice score considers local deviations from ground truth, whilst the manual QC and volume measurements capture global protocol deviations. The dice overlap by population is shown in Fig. 1, manual quality checks in Fig. 2 and volume comparison to ground truth in Fig. 3.
Conclusions: The presented CNN produces highly accurate, robust segmentations. In all metrics considered, the CNN outperforms an established technique. The CNN performance is shown consistent across several therapeutic areas – making it a promising tissue segmentation tool.
ENABLING CHARACTERISATION OF THE NAD+ BIOSYNTHESIS PATHWAY BY CEST MRI

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¹University of Warwick, Department Of Chemistry, Coventry, United Kingdom, ²University of Warwick, School Of Engineering, Coventry, United Kingdom, ³University of Warwick, Warwick Centre For Doctoral Training In Analytical Science, Coventry, United Kingdom, ⁴Molecular Imaging Center, Department Of Molecular Biotechnology And Health Sciences, Turin, Italy, ⁵University, Department Of Physics, Coventry, United Kingdom, ⁶Institute of Biostructures and Bioimaging (IBB), National Research Council Of Italy (cnr), Turin, Italy

Aims: The primary study aim was to characterise the Chemical Exchange Saturation Transfer (CEST) MRI properties of six metabolites required for synthesis of coenzyme nicotinamide adenine dinucleotide (NAD+) and to evaluate the potential for clinical imaging of altered NAD+ regulation in neurodegenerative disorders.

Methods: CEST images of metabolites in the NAD+ biosynthesis pathway were acquired at physiological temperature with high-field MRI (9.4T) running a modified magnetisation transfer (MTR) prepared RARE sequence over a range of saturation powers and frequency offsets, for pH values 5.5-7.4. CEST data were fitted using spline interpolation, the exchange rate was calculated, and frequency specific MTR maps were generated using pixelwise analysis.

Results: Tryptophan, nicotinamide, nicotinic acid (NA), nicotinamide riboside (NR), nicotinamide mononucleotide (NMN) and NADH were evaluated. At pH 7.4, all molecules exhibited CEST contrast between 0.1 and 0.9 ppm, originating from the OH hydroxyl proton. NADH exhibited up to 15% CEST contrast in a B¹ field of 2.4µT from its fast-exchanging amine groups at 2 and 3 ppm, and showed a positive dependence of CEST efficiency on pH. Tryptophan exhibited three independent peaks at 0.7, 2.6, and 5.4 ppm corresponding to pH 6.7, 5.5 and 7.4. NMN showed high CEST contrast at 0.7 ppm originating from its phosphorous OH enabling higher CEST efficiency due to enhanced charge delocalisation.
Conclusions: This in-vitro study characterized NADH and the metabolites of the NAD+ biosynthesis pathway at controlled pH, temperature, and concentration. The observed marked CEST contrast may support future non-invasive MRI-CEST imaging of NADH biosynthesis dysfunction in the context of neurodegenerative disorders.
Aims: To determine whether in vivo biomarkers of neuronal loss based on quantitative Gradient-Recalled-Echo (qGRE) MRI predict rates of cognitive decline better than total regional volume (atrophy).

Methods: We analyzed data from 55 participants (24 cognitively healthy, 17 with preclinical Alzheimer disease (AD), 14 with AD dementia) who had a qGRE scan and at least two annual cognitive assessments. In brain tissue, qGRE identifies two biomarkers, the proportion of tissue with low neuronal counts (Dark Matter Fraction, DMF) and the volume of tissue with viable neurons (Viable Tissue, VT). Linear mixed effects models were used to analyze the extent to which global cognitive decline was related to these measures compared to total hippocampal volume.

Results: DMF ($\beta = -0.11, p < .01$) and VT ($\beta = .10, p = .02$) correlated with cognitive decline whereas total hippocampal volume did not ($\beta = 0.07, p = 0.10$). Model comparisons using Akaike Information Criteria (AIC) indicated that a model incorporating DMF was more likely to predict out of sample data than a model that incorporated total volume by a factor of over 100 to 1. DMF outperformed VT by a factor of 11 to 1.

Conclusions: The DMF measured at baseline was significantly associated with decline in global cognition over 2+ years. Hippocampal volume did not predict decline over the same interval. DMF (and to a lesser extent, viable volume) may serve as a more sensitive marker of neurodegeneration than total hippocampal atrophy.
Aims: The purpose of this study was to compare visual rating scales with automated volumetry by icobrain dm and introduce an automated alternative for the MTA visual rating scale.

Methods: A total of fifty patients (65-90 years old) diagnosed with amnestic mild cognitive impairment (aMCI) or mild Alzheimer’s disease dementia (mild ADD) were enrolled in this study. Scheltens’ Medial temporal lobe atrophy (MTA), rated on coronal T1-weighted images, and Fazekas scores, rated on axial FLAIR images, were determined by an experienced radiologist (G-J. A.), who was blinded to all clinical information, including sex, age, and diagnosis. From each MRI scan, automated segmentations were computed by icobrain dm (v.5.1) for total (HIP-T), left (HIP-L) and right (HIP-R) hippocampal volumes, as well as for global, anterior and posterior white matter hyperintensities (WMH), including an automated alternative of the visual MTA score defined as the ratio between volumes of inferior lateral ventricle and hippocampus expressed as a percentage.

Results:

Study population characteristics are summarized in Table 1 and 2. There was a moderate negative correlation between MTA visual rating scores and hippocampal volumes (HIP-R: r=-0.5, p<0.001; HIP-L: r=-0.42, p=0.002; HIP-T: r=-0.49, p<0.001) (Figure 1). The Fazekas rating scales positively correlated with global (r=0.85, p<0.001), anterior (r=0.37, p=0.008) and posterior WMHs (r=0.75, p<0.001) (Figure 2). The automated MTA-alternative correlated strongly to visual MTA scores (r= 0.76, p<0.001) (Figure 3).
Table 1: Demographic and volumetric characteristics of the study population

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th># (%)</th>
<th>Mean (SD)</th>
<th>Median [Q1, Q3]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex - F (%)</td>
<td>29 (58.0%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Age</td>
<td>50</td>
<td>72.4 (6.2)</td>
<td>73.0 (67.3, 76.0)</td>
</tr>
<tr>
<td>MMSE</td>
<td>49</td>
<td>27 (2)</td>
<td>27 (26, 28)</td>
</tr>
<tr>
<td>Education (years)</td>
<td>50</td>
<td>12.4 (2.8)</td>
<td>12.0 (10.0, 15.0)</td>
</tr>
<tr>
<td>White matter hyperintensities (WMH)</td>
<td>50</td>
<td>11.262 (11.553)</td>
<td>5.873 (3.152, 16.082)</td>
</tr>
<tr>
<td>White matter hyperintensities - anterior (WMH-A)</td>
<td>50</td>
<td>4.205 (5.295)</td>
<td>2.214 (0.930, 4.699)</td>
</tr>
<tr>
<td>White matter hyperintensities - posterior (WMH-P)</td>
<td>50</td>
<td>7.057 (10.498)</td>
<td>2.349 (1.011, 7.336)</td>
</tr>
<tr>
<td>Hippocampal volume - total</td>
<td>50</td>
<td>7.842 (1.638)</td>
<td>8.183 (7.298, 8.926)</td>
</tr>
<tr>
<td>Hippocampal volume - left</td>
<td>50</td>
<td>3.813 (0.893)</td>
<td>4.120 (3.477, 4.361)</td>
</tr>
<tr>
<td>Hippocampal volume - right</td>
<td>50</td>
<td>4.029 (0.856)</td>
<td>4.265 (3.689, 4.532)</td>
</tr>
<tr>
<td>Fazekas – WMH lesions rating scale</td>
<td>50</td>
<td>1 (1)</td>
<td>1 (1, 2)</td>
</tr>
<tr>
<td>Medial temporal lobe atrophy (MTA) rating scale</td>
<td>50</td>
<td>1 (1)</td>
<td>1 (0.2)</td>
</tr>
</tbody>
</table>

Table 2: Percentage of participants per visual rating scale degree

<table>
<thead>
<tr>
<th>VISUAL RATING SCALE</th>
<th># (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fazekas – WMH lesions rating scale</td>
<td></td>
</tr>
<tr>
<td>• Fazekas = 0</td>
<td>3 (6.0%)</td>
</tr>
<tr>
<td>• Fazekas = 1</td>
<td>24 (48.0%)</td>
</tr>
<tr>
<td>• Fazekas = 2</td>
<td>16 (32.0%)</td>
</tr>
<tr>
<td>• Fazekas = 3</td>
<td>7 (14.0%)</td>
</tr>
<tr>
<td>Medial temporal lobe (MTA) rating scale</td>
<td></td>
</tr>
<tr>
<td>• MTA = 0</td>
<td>15 (30.0%)</td>
</tr>
<tr>
<td>• MTA = 1</td>
<td>20 (40.0%)</td>
</tr>
<tr>
<td>• MTA = 2</td>
<td>9 (18.0%)</td>
</tr>
<tr>
<td>• MTA = 3</td>
<td>3 (6.0%)</td>
</tr>
<tr>
<td>• MTA = 4</td>
<td>3 (6.0%)</td>
</tr>
</tbody>
</table>
Figure 1: Agreement between hippocampal volumes and MTA visual rating scale

Figure 2: Agreement between white matter hyperintensity volumes and Fazekas visual rating scale
Conclusions: Automated volumetry and visual scores showed good correlations for the assessment of medial temporal lobe atrophy and white matter hyperintensities in patients with amnestic MCI and mild ADD.
Aims: White matter alterations associated with Alzheimer’s disease (AD) have been much less studied than the changes in gray matter. In this study, we aim to identify altered white matter tracts connecting the four major lobes and hippocampus in patients with AD.

Methods: Based on diffusion-weighted images, tractograms were obtained with anatomical constrained tractography and with spherical-deconvolution informed filtering of tractograms in order to reconstruct realistic streamlines. We built connectivity matrices by determining the number of streamlines connecting every pair obtained from the following regions: the four major lobes (i.e., frontal, parietal, temporal, and occipital) and hippocampus. The statistical analysis was performed on 76 subjects, including 36 controls (age: $57.2 \pm 8.0$) and 40 patients (age: $72.2 \pm 10.6$) with AD. The number of streamlines linking pairs of regions was compared between both groups with a Student T-test and p-values were corrected for multiple comparisons.

Results: Our results showed that in patients with AD, the number of streamlines was significantly smaller between the hippocampus and the different lobes ($T$-value $<-3.5$; $p$-value $< 0.001$). In addition, altered connectivity was also observed between frontal and temporal lobes ($T$-value $=-3.7$; $p$-value=0.004), and frontal and occipital lobes ($T$-value $=-2.9$; $p$-value=0.04). It is also possible that these differences might be due to age.

Conclusions: Our study showed altered structural connectivity between regions typically associated with AD, in particular the hippocampus. In addition to hippocampal volume loss, white matter alterations in the hippocampus can be a potential biomarker for assessing the clinical status of patients with AD.
CORTICAL THICKNESS IS RELATED TO COGNITIVE-MOTOR AUTOMATICITY IN INDIVIDUALS WITH ALZHEIMER’S DISEASE: A REGIONS OF INTEREST STUDY

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Aims: Alzheimer’s disease (AD) is characterized by a distinct pattern of cortical thinning and resultant changes in cognition and function. These result in prominent deficits in cognitive-motor automaticity. The relationship between AD-related cortical thinning and decreased automaticity is not well understood. We aimed to investigate the relationship between cortical thickness regions-of-interest and automaticity in AD using both hypothesis-driven and exploratory approaches.

Methods: We performed a regions-of-interest (ROI) analysis of 46 patients with AD. Data regarding MRI images, demographic characteristics, cognitive-motor dual-task performance, and cognition were extracted from medical records. Cortical thickness was calculated from MRI images using FreeSurfer. Data from the dual-task assessment was used to calculate the combined dual-task effect, a measure of cognitive-motor automaticity. Two hierarchical multiple linear regression models were conducted on 1) hypothesis-generated ROIs and 2) exploratory ROIs.

Results: The hypothesis-driven and data-driven models each consisted of four ROIs, and differed from each other by only one brain region. Overall hypothesis-driven and data-driven ROIs explained 21.5% (p=.010) and 24.8% (p=.003) variability in automaticity, respectively. The dorsal lateral prefrontal cortex and superior parietal cortex were significant predictors of automaticity (p≤.011).

Conclusions: Cortical thinning in AD was related to cognitive-motor automaticity, particularly in the dorsal lateral prefrontal and superior parietal cortices. This suggests that these regions may play a primary role in automaticity. Further research is warranted.
Aims: OBJECTIVES: Alzheimer Disease (AD) and Frontotemporal Dementia (FTD) are characterized by progressive brain atrophy at variable rates along the age continuum. We suggest using normative templates according to cortical thickness (CTh) within different age ranges.

Methods: METHODS: We studied 497 MRIs of healthy controls (CTR), Early Onset AD (EOAD), Late Onset AD (LOAD) and FTD patients. Subjects were grouped according to age: [45-54 years] (21 CTR, 17 EOAD, 13 FTD), [55-64 years] (93 CTR, 77 EOAD, 63 FTD), [65-74 years] (73 CTR, 7 EOAD, 43 LOAD, 37 FTD) and [75-84 years] (15 CTR, 28 LOAD, 10 FTD). We obtained regional CTh and we generated group-normative maps using the mean and standard deviation (SD) of CTR. Then, Z scores were estimated as $Z_{region} = \frac{CTh_{region} - CTh_{CTRmean}}{CTh_{CTRSD}}$ for each patient with its corresponding template. We compared these maps as well as global values at each age range.

Results: RESULTS: AD and FTD showed brain atrophy compared to CTR at all age groups. The effects of atrophy for both diseases with respect to CTR were stronger at younger ages (Figure 1). We describe differences between AD and FTD along the aging continuum with different patterns across ages (Figure 2).
Conclusions: CONCLUSION: We highlight the necessity of using age-matched templates to identify changes across the disease timeline for AD and FTD. At younger ages, EOAD and FTD had partly overlapping brain signatures. At older ages, LOAD and FTD clearly show a different pattern. These patterns can be used to support the differential diagnosis of these dementias.
Aims: ALK6021-201 was a Phase 2, randomized, placebo-controlled clinical trial to assess the safety and tolerability of GRF6021, a plasma-derived product, in patients with Parkinson's disease and cognitive impairment. Magnetic resonance imaging (MRI) biomarkers were assessed as an exploratory investigation of therapeutic efficacy.

Methods: A subset of 37 patients randomized to placebo or GRF6021 infusions completed MRI evaluations at baseline and day 90 follow-up. A standardized protocol was used to acquire volumetric, functional, and arterial spin labeling sequences across multiple centers. FreeSurfer was used to parcellate the 3DT1, and longitudinal change was assessed using tensor-based morphometry. Resting-state connectivity was assessed via temporal cross-correlation between anatomic and functional nodes. Regional cerebral blood flow (CBF) was quantified following M0 calibration.

Results: After 90 days, regional atrophy rates were similar between treatment groups, with marginally less entorhinal cortex thinning in the GRF6021 group (p=0.38). The GRF6021 group also demonstrated longitudinal increases in functional connectivity between executive, sensorimotor, and frontoparietal networks (p<0.004 to 0.05). Improved vascular function was also detected, as patients treated with GRF6021 showed an increase in CBF in frontal (p=0.3), parietal (p=0.07), and occipital (p=0.2) lobes, while CBF in these regions declined in the placebo group (p=0.1, 0.3, and 0.001).

Conclusions: Repeat infusions of GRF6021 for 5 consecutive days approximately every 3 months was associated with structural, functional, and vascular MRI biomarker improvements compared to placebo when assessed at day 90 follow-up.
DIFFERENCES IN DYNAMIC FUNCTIONAL CONNECTIVITY ACROSS THE ALZHEIMER’S DISEASE SPECTRUM

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Aims: To examine differences in dynamic functional connectivity (DFC) in cognitively normal subjects (CN), and patients with mild cognitive impairment (MCI) or Alzheimer’s disease (AD).

Methods: Resting-state functional magnetic resonance imaging (rsfMRI data of 76 participants (41 CN, 24 MCI, 11 AD) from the ADNI database (http://adni.loni.usc.edu/) was used for analysis. Groups were matched according to age, sex and education. rsfMRI data were pre-processed using the CONN toolbox. For data reduction purposes, spatial independent and principal component analyses were performed on the normalized rsfMRI using GIFT. Resulting meaningful independent components were clustered into 13 functional resting-state networks. Using GIFT, a sliding window approach was employed to determine dynamic functional connectivity states across the scan period of 9 minutes. Afterwards, non-parametric t-tests were performed comparing the groups in measures of DFC such as dwell time (i.e. staying in a state), number of transitions (i.e. switching between states) and fraction time (i.e. total time spent in a state).

Results: The DFC analysis resulted in 4 distinct functional connectivity states, which were characterized by either global connectivity patterns or distinct connectivity patterns between certain rs-networks. Group comparisons yielded that one state was particularly more occupied by the AD group compared to the CN group (p = .02) and the MCI group (p = .001).

Conclusions: Network reorganization may influence dynamic functional connectivity patterns in pre- and clinical AD.
AN IMPACT OF APOE E4 ALLELE ON TRIMODAL ASSOCIATION BETWEEN SUBTHRESHOLD, POSITIVE AMYLOID-B RETENTION, FUNCTIONAL CONNECTIVITY, AND COGNITIVE FUNCTION IN COGNITIVELY UNIMPAIRED OLDER ADULTS

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Aims: Although previous studies demonstrated the Aβ-dependent pathway of the APOE ε4 allele, research has yet to systematically investigate the impact of the APOE ε4 allele on the pathway from the sub- and above-threshold Aβ burden to cognitive function in the preclinical phase. In this regard, this study aimed to explore the effect of the APOE ε4 allele on the trimodal association between Aβ retention, brain function, and cognitive performances in older adults with intact cognition, depending on the Aβ burden status.

Methods: 182 older adults with normal cognition underwent functional MRI and were dichotomized using \([^{18}\text{F}]\)-labelled flutemetamol PET into subjects with sub-threshold [CN sub-Aβ, n = 110; (APOE ε4 carrier, n = 30; non-carrier, n = 80)] and positive Aβ deposits [CN Aβ+ group, n = 72; (APOE ε4 carrier, n = 34; non-carrier, n = 38)].

Results: This study found that (i) the affected cognitive function differed according to the degree of Aβ retention in the APOE ε4 carrier; (ii) the difference in the local functional connectivity (FC) was found in the brain regions of the default-mode network and salience network according to the APOE ε4 allele; (iii) the differential association between regional Aβ retention and local connectivity according to the APOE ε4 allele was found in each CN sub-Aβ and Aβ+ group, depending on the degree of the Aβ burden.

Conclusions: This study has been the first attempt to thoroughly examine the mechanism at play in the earliest phase of Alzheimer’s disease, considering both the Aβ retention and APOE ε4 allele.
AMYLOID BETA DEPOSITION AND COGNITIVE DECLINE IN PARKINSON’S DISEASE: A STUDY OF THE PPMI COHORT

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Aims: To better understand the relationship between Parkinson’s disease (PD) cognitive decline and amyloid beta (Aβ) deposition, our study focused on a homogenous group of idiopathic PD patients whose cognitive abilities were measured longitudinally for two years after their Aβ scan.

Methods: Our study used PD patient and healthy control (HC) data from the Parkinson’s Progression Marker Initiative (PPMI) cohort. 25 de novo idiopathic PD patients and 31 HC underwent a [18F]florbetaben (FBB) positron emission tomography (PET) scan which can measure the density of Aβ in vivo in the brain. Demographic information, clinical characteristics, and cognitive test scores were also collected. The data was analyzed using IBM SPSS version 27 software with linear regression modeling, Pearson correlations, and hierarchical cluster analysis.

Results: A stepwise linear regression of the PD group revealed a strong adjusted R² of 0.495 in a model explaining their Montreal Cognitive Assessment (MoCA) score 1-year post-scan using the SUVR from 20 cortical ROIs as independent variables. The ROIs found in this stepwise model were the left rectus, the left anterior cingulate cortex, and the right parietal cortex. We found the PD group’s brain regions formed two clusters, with increased Aβ in cluster two correlating more strongly with a lower MoCA.

Conclusions: The results suggest Aβ has a moderate association with cognitive decline in PD. We found Aβ accumulation in the brain had a patchwork effect on PD cognition depending on which ROIs are affected. More Aβ accumulation in cluster 2 ROIs may be involved in PD patients’ cognitive dysfunction.
POSTERS

AN AUTOMATED PIPELINE FOR CENTILOID QUANTIFICATION OF AMYLOID LOAD USING MULTIPLE PET TRACERS

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Aims: The Centiloid scale was introduced to standardize in vivo quantitative amyloid plaque estimation by Positron Emission Tomography (PET). The purpose of this study was to develop a single and fully automatized Centiloid quantification pipeline for multiple amyloid PET compounds.

Methods: Our fully automated SPM12 pipeline was validated on PiB-PET scans of Young Controls (YC) and Alzheimer’s Disease (AD) patients (N = 79) and F18-PET scans (Florbetapir: N=46; Florbetaben: N=35; Flutemetamol: N=74; NAV4694: N=55), from the Centiloid project. Then, correlations of (PiB-, F18-) SUVr values and published SUVr data were computed. Further, correlations between F18 SUVr and paired PiB SUVr were computed. Correlation coefficients (R²) > 0.7 were required to consider the Centiloid calibration valid.

Results: Validation results were within the bounds defined by the Centiloid method (SUVr_AD-100 = 2.08 +/- 0.2; SUVr_YC-0 = 1.01 +/- 0.05; R2 = 1.00; slope = 1.00; intercept = -0.1) Florbetapir, Florbetaben, Flutemetamol, NAV4694 and PiB SUVr correlation coefficients with published values were all above 0.99. Correlation coefficients of F18 SUVr with PiB SUVr were respectively 0.91, 0.95, 0.96, 0.99 which is well above the recommended value (0.7). Equations for converting to Centiloid were respectively: CL = 177.79 FBP_SUVr – 183.56 CL = 153.08 FBB_SUVr – 152.93 CL = 122.39 FTM_SUVr – 120.97 CL = 90.20 NAV_SUVr – 91.61

Conclusions: We demonstrate the reliability of a fully automated amyloid PET pipeline for multiple amyloid-PET compounds (PiB and F18) suitable for implementation in clinical trials.
Aims: Antibodies which pass the blood-brain barrier (BBB) via transferrin (TfR) receptor-mediated transcytosis could be useful in molecular imaging. However, the long biological half-life of IgG antibodies makes radiolabelling with short-lived radioisotopes unsuitable. To solve this issue, a pre-injected BBB penetrating antibody, labelled with a transyclooctene (TCO) tag, can be targeted with a radiolabelled tetrazine. This pretargeted approach, based on in vivo bio-orthogonal tetrazine-TCO ligation, requires the development of a tetrazine which can enter the brain. The aim for our study was to synthesise different $^{18}$F-tetrazines as pretargeting agents and assess their BBB penetration and pharmacokinetics.

Methods: A two-step synthesis method was developed to label two novel tetrazine via $^{18}$F-Py-TFP synthesized on solid support followed by amidation with amine/aminooxy-bearing tetrazines. Each radiolabelled tetrazine was injected into two transgenic (tg-ArcSwe) and two wild-type mice, to assess its brain pharmacokinetics via PET imaging and organ extraction. A published $^{18}$F-tetrazine served as a comparison.

Results: Radiolabelling of the tetrazines resulted in radioactivity yields of 590 ± 330 MBq and a radiochemical purity of > 93%. All three fluorine-18 labelled methyltetrazine radiotracers overcame the BBB and displayed a concentration maximum in the brain within the first minute. Time-activity curves from PET-scans showed all tetrazines entered the brain efficiently, but the average SUV after one hour scan remained high for $^{18}$F1 (SUV = 0.96) compared to $^{18}$F2 (SUV= 0.59) and $^{18}$F3 (SUV=0.29).

Conclusions: Here we could present two new tetrazines which will be used for future investigations in neuroimaging, like in vivo pre-targeting.
INCREASED PLASMA GLIAL FIBRILLARY ACIDIC PROTEIN IS ASSOCIATED WITH HIGHER CEREBRAL GLUCOSE CONSUMPTION EARLY IN THE ALZHEIMER'S CONTINUUM

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Aims: Glial fibrillary acidic protein (GFAP) is a marker of reactive astrogliosis that can be measured in both blood and cerebrospinal fluid (CSF). Plasma GFAP has been suggested to become altered earlier in Alzheimer's disease (AD) than its CSF counterpart. Although astrocytes consume approximately half of the glucose-derived energy in the brain, the relationship between reactive astrogliosis and cerebral glucose metabolism is poorly understood. Here, we aimed to investigate the association between fluorodeoxyglucose ([¹⁸F]FDG) uptake (glucose-metabolism marker) and GFAP, quantified in both plasma and CSF for the same participants.

Methods: We included 314 cognitively unimpaired participants from the ALFA+ cohort, 112 of whom were amyloid-β (Aβ) positive. Associations between GFAP markers and CSF Aβ42/40 and global/regional [¹⁸F]FDG uptake (glucose-metabolism marker) and GFAP, quantified in both plasma and CSF for the same participants.

Results: Both GFAP markers were increased with higher Aβ pathology (Figure 1). Plasma GFAP was positively associated with glucose consumption in the whole brain; this association was stronger and in different areas compared to CSF GFAP (Figure 2). Furthermore, this association became negative in Aβ- and tau-positive participants (A+T+) in similar areas of AD-related hypometabolism (Figure 3).

Conclusions: Our findings suggest that higher astrocytic reactivity, probably in response to early AD pathological changes, is related to significantly higher glucose consumption. With the onset of tau pathology, the observed uncoupling between astrocytic biomarkers and glucose consumption might be indicative of a failure to sustain the higher energetic demands required by reactive astrocytes, that are already in preclinical AD.
Figure 1: Associations between GFAP biomarkers and CSF Aβ42/40
The first and second columns show the negative associations between CSF Aβ42/40 and plasma (A) and CSF GFAP (B), respectively. Given that Aβ pathology increases with lower CSF Aβ42/40 levels, these figures show that higher GFAP levels, both measured in plasma and in CSF, were related with higher Aβ pathology. Both plasma and CSF GFAP were quantified on a Simoa HD-X (Quanterix, Billerica, MA, USA) using the commercial single-plex assay (#102336). Aβ42 and Aβ40 were measured with the exploratory Roche NeuroToolKit immunoassays (Roche Diagnostics International Ltd) on a cobas e 411 analyzer or cobas e 601 modules.
Abbreviations: Aβ, amyloid-β; CSF, cerebrospinal fluid; GFAP, glial fibrillary acidic protein.
Figure 2: Associations between GFAP biomarkers and \(^{18}\)F\( \text{FDG} \) uptake

The first column depicts the specific association between adjusted global \(^{18}\)F\( \text{FDG} \) uptake and plasma (A) and CSF GFAP (B), respectively. The second column shows the areas of the brain with a significant positive association between plasma (A) and CSF GFAP (B) and the regional \(^{18}\)F\( \text{FDG} \) uptake using a voxel-wise approach. Both plasma and CSF GFAP were quantified on a Simoa HD-X (Quanterix, Billerica, MA, USA) using the commercial single-plex assay (#10236).

Abbreviations: CSF, cerebrospinal fluid; \(^{18}\)F\( \text{FDG} \), \(^{18}\)F-fluorodeoxyglucose; GFAP, glial fibrillary acidic protein; LI, left inferior; LL, left lateral; LM, left medial; RI, right inferior; RL, right lateral; RM, right medial; SUV, standardized uptake value ratio.
Figure 3: Interaction effect of GFAP biomarkers and AT stages on $[^{18}F]$FDG uptake

The first column shows the areas where participants in A-T and A+T groups have a significantly different association than participants in A+T group between $[^{18}F]$FDG uptake and plasma (A) and CSF GFAP (B) biomarkers, respectively. The second column depicts the specific association between adjusted $[^{18}F]$FDG uptake in the cluster marked with an asterisk (*) and each of the GFAP biomarkers by AT stages. These areas were bilateral thalami (A) and bilateral precuneus (B), respectively. AT groups were derived from previously published thresholds on CSF Aβ42/40 (0.071) and p-tau (24 pg/ml). Both plasma and CSF GFAP were quantified on a Simoa HD-X (Quanterix, Billerica, MA, USA) using the commercial single-plex assay (#102336). CSF p-tau was measured using the electrochemiluminescence Elecsys® Phospho-Tau(181P) CSF immunoassay and on a fully automated cobas e 601 instruments (Roche Diagnostics International Ltd). Aβ42 and Aβ40 were measured with the exploratory Roche NeuroToolKit immunoassays (Roche Diagnostics International Ltd) on a cobas e 411 analyzer or cobas e 601 modules.

Abbreviations: Aβ, amyloid-β; A-T, Aβ-negative tau-negative; A+T, Aβ-positive tau-negative; A+T+, Aβ-positive tau-positive; CSF, cerebrospinal fluid; $[^{18}F]$FDG, $[^{18}F]$fluorodeoxyglucose; GFAP, glial fibrillary acidic protein; LI, left inferior; LL, left lateral; LM, left medial; p<0.001; p-value from the interaction effect; p-tau, phosphorylated tau; RI, right inferior; RL, right lateral; RM, right medial; SUVR, standardized uptake value ratio.
EXPLORING THE BRAIN METABOLISM OF THE TGF344 RAT MODEL OF ALZHEIMER’S DISEASE

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Aims: Animal models are key to understanding neuropathologies and assessing new therapies. Therefore, it is crucial to ensure that they capture the complexities of human diseases. In Alzheimer’s disease (AD), the TGF344 rat model has been widely characterized, but the potential changes in brain glucose metabolism at middle stage (one of the hallmarks of AD) has never been explored. This study aims to fill this gap.

Methods: Sixteen-month-old TGF344-AD rats (n=12) and wild type (WT, n=12) littermates of both sexes were evaluated with a 20-minute static [¹⁸F]FDG-PET acquisition, performed 40 minutes after intravenous [¹⁸F]FDG injection. The images were processed with PMOD software, and the radiotracer uptake was quantified using the standardized uptake value normalized by the blood glucose levels (SUVglu). Statistical analyses were performed with GraphPad Prism 9.2.

Results: TGF344-AD rats presented a trend of reduced blood glucose levels compared to WT (8.22 ± 0.95 vs 9.01 ± 0.98 mmol/l, p = 0.057). Similarly, the analysis of glucose metabolism (29 brain regions, W. Schiffer atlas) suggested reduced SUVglu estimates in AD animals, with the largest mean group differences in the pituitary (21.8%), midbrain (10.7%), ventral tegmental area (10.8%), and hypothalamus (8.7%). However, these differences were not significant (p > 0.05).

Conclusions: Despite presenting slightly lower SUVglu levels, our results suggest that TGF344-AD and WT animals have similar brain glucose metabolization at 16 months of age. These results may be explained by the microgliosis and astrogliosis present at this stage, and these cells’ role in glucose metabolism. Immunohistochemistry analyses are ongoing to test this hypothesis.

Figure 1. Glucose metabolism in sixteen-month-old WT and TGF344 rats of both sexes. A) The plot represent the blood glucose levels in wild-type (WT) and TGF344-AD animals of both sexes. B) Representative 20-min static [¹⁸F]FDG images of one WT and one TGF344-AD animals. C) [¹⁸F]FDG uptake in 29 brain regions, represented as the mean SUVglu (standardized uptake value normalized by the blood glucose level) per group. Error bars represent the standard error of the mean. WT: n = 12, TGF344: n = 12

Conclusions: Despite presenting slightly lower SUVglu levels, our results suggest that TGF344-AD and WT animals have similar brain glucose metabolization at 16 months of age. These results may be explained by the microgliosis and astrogliosis present at this stage, and these cells’ role in glucose metabolism. Immunohistochemistry analyses are ongoing to test this hypothesis.
EVALUATION OF TAU DEPOSITION IN AMYLOID-POSITIVE MCI AND MILD-AD DEMENTIA
SUBJECTS FROM THE MISSIONAD PROGRAM USING 18F-PI-2620

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Aims: To characterize tau deposition using 18F-PI-2620 PET tracer in amyloid-positive MCI or mild AD dementia patients with respect to amyloid deposition, cerebrospinal fluid (CSF) biomarkers, hippocampal volume and neurocognitive assessment.

Methods: Placebo-treated, amyloid-positive patients with a diagnosis of MCI due to AD or mild AD dementia from the elenbecestat MissionAD Phase 3 program (n=74, 76 ± 7 yrs, 38 females) underwent a baseline 18F-PI-2620 PET, T1-weighted MRI, and several cognitive tests. A subset of participants underwent CSF assessment (Aβ42-Aβ40 ratio, p-tau, t-tau; n=22) and a one-year follow-up 18F-PI-2620 PET scan and cognitive assessments (n=13).

Results: Tau scans were more often positive in patients with increased amyloid-beta deposition (7.7% (<36 CL) vs. 80% (>83 CL)). Elevated PI-2620 SUVR was associated to high p-tau and t-tau (p=0.0006 and p=0.01 (fusiform gyrus)) in CSF. Low hippocampal volume was associated with increased tau load at baseline. Longitudinally, significant increases in tau load were observed in the mesial temporal cortex, fusiform gyrus, and inferior temporal cortex. The MMSE (recall score), ADAS-Cog (word recognition score), and CBB (one-card learning score) showed the strongest association with tau deposition at baseline with consistent p-values below 0.05 (without correction for multiple comparisons) in the mesial temporal, fusiform gyrus, and inferior temporal cortex.

Conclusions: This study supports the utility of 18F-PI-2620 PET to assess tau deposits in an early AD population showing significant correlations with established structural and CSF biomarkers and inverse correlations with cognitive scores in domain-specific patterns.
Aims: To develop a carbon-11 labelled PET radiotracer - \([^{11}C]IF1\) targeting glutamate transporters; To evaluate the biodistribution of \([^{11}C]IF1\) in mice using microPET imaging;

Methods: The Eckert & Ziegler Modular-Lab system was chosen for carrying out the \([^{11}C]IF1\) radiosynthesis. Cyclotron-derived \([^{11}C]CO_2\) was reduced to \([^{11}C]CO\) as described by Taddei et al. (2015). The \([^{11}C]CO\) afforded \([^{11}C]IF0\) via a palladium-mediated \([^{11}C]\)carbonylation reaction. \([^{11}C]IF1\) was obtained after removal of the BOC protecting group with trifluoroacetic acid. Positron emission tomography (PET) imaging was conducted in healthy adult mice. Following anaesthesia (isoflurane 2.5%), \([^{11}C]IF1\) in saline solution (ethanol 2%) was injected in the mouse tail vein. The biodistribution was obtained using dynamic PET scan (60 min). Immunohistochemistry was also conducted to evaluate the presence of glutamate transporters in the periphery.

Results: We obtained \([^{11}C]IF1\) within 18 min after the end of bombardment. The radio-HPLC analysis of the crude product resulted in radiochemical yield of 79%. Reformulated \([^{11}C]IF1\) in saline solution (ethanol 2%) had radiochemical purity of 99%. Our preliminary PET imaging analysis showed high standardize uptake values (SUVs) in the intestine (SUV = 17) and liver (SUV = 6), but low in the brain (SUV = 0.8). Immunohistochemistry analysis demonstrated that the intestine is enriched in glutamate transporters, which co-localise with neuronal cells of the enteric nervous system.

Conclusions: \([^{11}C]IF1\) was synthesised and seems to bind to glutamate transporters, which are impaired in AD. Work is in progress to further characterise \([^{11}C]IF1\) peripheral binding and to optimize this reaction, furnishing a pharmacophore capable of penetrating the BBB.
WHOLE-BODY SPECT IMAGING OF GLYMPHATIC INFLUX AND EFFLUX IN A MOUSE MODEL OF BETA-AMYLOIDOSIS

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Aims: Protein accumulation and ultimate deposition in the brain is the pathological hallmark in patients with Alzheimer’s disease, Parkinson’s disease and other neurodegenerative diseases implying that a reduced waste clearance from the brain into the periphery is a common feature in neurodegeneration. The glymphatic system is a recently described pathway that clears the brain parenchyma of toxic proteinaceous metabolites, including the amyloid-beta protein, via convective influx of cerebrospinal fluid into the brain parenchyma followed by perivenous efflux of interstitial fluid into the periphery. Although initially described in the rodent brain, clinical studies could verify that CSF flow patterns in humans resemble the glymphatic flow in rodents. Furthermore, these clinical studies suggest that CSF clearance is reduced in patients with Alzheimer’s disease.

Methods: To assess glymphatic influx and efflux pathways in health and neurodegeneration, we introduce whole-body SPECT scanning combined with computed tomography for the dynamic imaging of glymphatic flow in a mouse model of beta-amyloidosis at different states of protein deposition and age-matched non-transgenic littermates.

Results: Preliminary results indicate that both glymphatic influx into the brain as well as efflux from the brain into the body periphery are diminished in the mouse model of beta-amyloidosis prior to histologically visible protein deposition in the brain parenchyma. Currently, we are aiming to correlate the reduction in glymphatic flow with increased concentrations of beta-amyloid, astrocyte and microglia activation as well as biomarkers for neurodegeneration.

Conclusions: These tools will now allow us to study the importance of glymphatic fluid flow in health and neurodegeneration.
MAGNETIC RESONANCE SPECTROSCOPIC IMAGING ENABLES SPATIAL MAPPING OF DECREASED GLUTAMATE LEVELS ASSOCIATED WITH TAU DEPOSITIONS IN ALZHEIMER’S DISEASE BRAINS

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Aims: Despite accumulating evidence for impaired glutamatergic neurotransmission in Alzheimer’s disease (AD) brains, its significance in neurofunctional and neuropathological alterations remains elusive. The aim of this study is to evaluate the associations between glutamatergic dysfunctions and tau depositions across cortical regions in AD patients using magnetic resonance spectroscopic imaging (MRSI).

Methods: We enrolled 16 patients with AD, consisting of cases with mild cognitive impairment due to AD and AD dementia, and 15 healthy controls (HCs). We performed tau and amyloid-β (Aβ) PET with ¹⁸F-PM-PBB3 and ¹¹C-PiB, respectively, and single-plane MRSI for evaluating a glutamate/creatine (Glu/Cr) ratio at the level of the cingulate gyrus. PET probe retentions were quantified as standardized uptake value ratio (SUVR) using the cerebellar cortex as a reference region.

Results:
Figure 1. A representative PET image showing $^{18}$F-PM-PBB3 and $^{11}$C-PiB SUVR superimposed on an individual MRI data and MRSI voxels.
Figure 2. A Z-score heatmap for the difference in $^{18}$F-PM-PBB3 and $^{11}$C-PiB SUVR between the AD and HC groups in MRSI voxels.
Figure 3. A Z-score heatmap for the difference in Glu/Cr between the AD and HC groups in MRSI voxels.
Z-score maps of the AD group compared to the HC group showed marked tau and Aβ depositions in extensive cortical gray matter regions, and reduced glutamate levels in more confined areas (Figures 1-3). Glutamate levels were correlated with tau but not Aβ burdens in some regions, including the posterior cingulate cortex (PCC) (Figures 4). In an analysis of combined voxels covering PCC, Glu/Cr ratios were correlated negatively with tau deposits in the AD group ($r = -0.53$, $p < 0.05$) and positively with mini-mental state examination scores ($r = 0.74$, $p < 0.05$) in AD dementia cases.

**Conclusions:** MRSI revealed the regionally variable vulnerability of the glutamatergic system to tau depositions in AD brains. In PCC, tau accumulations are likely to induce disrupted glutamine transmissions, aggravating cognitive functions.
DIFFERENT REGIONAL PATTERNS OF CORTICAL MICROSTRUCTURAL ALTERATIONS IN ALZHEIMER’S DISEASE, PRIMARY PROGRESSIVE APHASIA, DEMENTIA WITH LEWY BODIES AND VASCULAR DEMENTIA.

M. Torso, G. Ridgway, M. Bozzali, I. Hardingham, S. Chance

Aims: The aim of the present study was to investigate the cortical microstructural signature in different dementia forms, using novel Diffusion Tensor Imaging measures.

Methods: Seventy-two participants with dementias [25=Alzheimer’s Disease (AD); 20= Primary Progressive Aphasia (PPA); 17= Vascular Dementia (VD); 10= Dementia with Lewy bodies (DLB)] and 22 cognitively normal (CN) volunteers were included in the present study. T1 structural and diffusion tensor imaging (DTI) scans were analysed to calculate 3 novel cortical grey matter diffusion measures (AngleR, PerpPD and ParlPD) [McKavanagh et al., 2019; Torso et al. 2020] and the mean diffusivity (MD). Values from sixty-eight cortical regions were used to investigate the cortical microstructural signature for each Diagnostic group compared with the cognitively normal group. Results were considered statistically significant after false discovery rate correction (FDR).

Results: The results showed that the cortical diffusivity measures detected group differences and PerpPD values, in particular, detected altered quality of cortical grey matter in all patient groups. Each diagnostic group showed a specific pattern of cortical microstructural alterations (Figure 1).

Conclusions: The regional analysis revealed the presence of microstructural alterations in key regions commonly affected in the diagnostic groups included in the study. These findings can support the use of
cortical diffusivity measurements as a surrogate of cortical microstructure quality for identification of neurodegenerative diseases and enabling differential diagnosis.
EVALUATE THE UTILITY OF SAPPHIRE II SCANNING COMPARED TO QUANTITATIVE BETA AMYLOID PET SCANS IN MCI AND MILD AD SUBJECTS.

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Aims: Objectives: To compare data from an innovative drug-device combination eye scanner (SAPPHIRE II) that detects Alzheimer's Disease (AD)-specific β-amyloid pathology in the ocular lens to quantitative beta amyloid PET scans in MCI and mild AD subjects.

Methods: The SAPPHIRE II system combines a fluorescent ligand (Aftobetin) ointment formulated as a medical imaging ointment, applied to the lower eye lid, and a fluorescent laser eye scanning device (FLES). Thirty four patients (24 MCI and 10 mild AD) underwent eye scanning, cognitive testing and amyloid PET scans. FLES scores were compared to PET SUVr and ROC curves were used to determine which SUVr and FLES cutoff values were most effective.

Results: No serious adverse events were reported. The ideal range for FLES was found to be between 0.887 and 0.912 with a SUVr cutoff of 1.12, yielding an AUC of 0.70. Though there were few subjects with low FLES and low SUVr scores or high FLES and high SUVr scores, a significant relationship was observed with 70.6% congruency in FLES and SUVr scores. Cutoff scenarios explored were FLES=0.883, 0.887, or 0.912 compared with PET SUVr=1.12. A FLES cutoff of 0.912 achieved a reasonable balance of sensitivity (74%) to specificity (73%) with 74% accuracy, while yielding a high PPV (85%) and reasonable NPV (57%).

Conclusions: Conclusion: These data from Cognoptix' SAPPHIRE II system suggest there is good correlation of AD-associated
Aims: Many immunotherapeutic clinical trials for Alzheimer's disease (AD) have failed due to poor translatability of promising transgenic mouse data to humans. The squirrel monkey (SQM) is a unique non-human primate (NHP) model that develops extensive age-associated cerebral amyloid angiopathy (CAA). Given the prominence of CAA in human AD cases and the critical need for a more proximate model of age-associated AD pathology, our study aimed to evaluate the feasibility of neuroimaging methodologies to characterize the biology of aging and disease in SQMs.

Methods: Multi-gradient echo (MGE) R2* maps were compared across age groups (5, 21 y/o) for brain pathology differences. Ex-vivo diffusion kurtosis imaging (DKI) was tested for the first time to evaluate brain tissue integrity and white matter (WM) microstructure, followed by postmortem immunohistochemistry to investigate underlying pathology of imaging abnormalities.

Results: MGE analysis successfully distinguished a 5 y/o SQM with no amyloid deposits from a 21 y/o with advanced pathology. We also observed age-associated increases in R2* values in all defined cortical brain regions in the longitudinal study. ARIA-E-like T2-w signal hyperintensities were detected in our oldest cohort. Ex-vivo DKI showed strongly increased mean kurtosis in a monkey with an ARIA-E like lesion. Preliminary assessment of matched histological regions to DKI abnormalities revealed WM demyelination, microgliosis, and blood-brain-barrier breakdown.

Conclusions: The current study highlights the utility of MRI methodologies to monitor age-dependent progression of Aβ-related pathology, as well as indicates that DKI can serve as a sensitive biomarker for brain microstructural changes and disease progression in our SQM model of CAA.
LABEL-FREE DETECTION OF ABETA PLAQUES - A DEEP LEARNING APPROACH TO INFRARED MICROSCOPY OF AD BRAIN TISSUE

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Aims: Alzheimer's disease (AD) is neuropathologically characterized by the deposition of amyloid beta (Aβ) plaques and neurofibrillary tangles within the brain. Aβ plaques are routinely recognized by their morphology in immunohistochemistry (IHC) images. However, staining procedures hinder the downstream analysis of samples and the development of an objective and universal plaque classification.

Methods: Building on recent work by (Röhr et al., 2020), we performed Infrared (IR) Imaging on fresh-frozen post-mortem brain tissue sections from AD cases and healthy control cases. The same tissue samples were subsequently stained against Aβ. IHC and IR images were aligned using a dedicated image registration approach. A convolutional neural network (CNN) was trained and validated that uses coarse-grained sample labels (Aβ-positive vs. Aβ-negative) to infer the pixel-precise location of plaques.

Results: The trained CNN infers an activation map of pathological regions in a given sample. Combined with a suitable threshold, the CNN detects Aβ plaques with high precision within entire thin-sections of human AD brain tissue.

Conclusions: The application of deep learning to label-free IR spectroscopic images of brain tissue yields a segmenting classifier that detects and precisely localizes AD plaques. The associated infrared spectra yield a biochemical fingerprint that may guide further pathological subclassification of Aβ plaques as well as subsequent molecular investigations, promising to further unravel the precise role of Aβ plaques in AD. References: Röhr, Dominik, Baayla DC Boon, Martin Schuler, Kristin Kremer, Jeroen JM Hoozemans, Femke H. Bouwman, Samir F. El-Mashtoly et al., Acta neuropathologica communications 8, no. 1 (2020): 1-13.
Aims: Within the central nervous system, microglia provide immune surveillance to the local tissue environment, undergoing rapid shifts toward responsive states based on diverse stimuli. Functional adjustments of microglia are accompanied by complex morphological changes that are challenging to quantify and interpret using conventional image segmentation and morphometric approaches. To dissect the complexity of microglial morphology, we developed a pipeline to 1) robustly segment microglia from 3D images of brain tissue and 2) generate label-free classification of microglial states from morphometric features in mouse brains.

Methods: Individual microglial cell bodies and soma were segmented in 3D using a convolutional neural network. A large bank of morphological descriptors were calculated, including skeletal, fractal, and Sholl-like features, across a data set comprising thousands of microglia. Dimensionality reduction and clustering techniques assigned phenotypic states to each cell, representing a continuum from resting through activated microglia.

Results: Deep learning-based segmentation successfully segmented microglia in a wide variety of configurations, densities, and shapes, and was able to decompose microglia into soma and processes. Morphological profiling grouped a wide variety of cell shapes into phenotypic categories without human supplied labels, suggesting those states are inherent to microglial biology.

Conclusions: For the first time, a combination of fully 3D deep-learning based segmentation, morphologic profiling, and phenotypic clustering was used to quantify morphometric alterations of microglia. This novel analytical pipeline could have broad application to study microglia phenotyping in response to treatment and disease in preclinical models.
RELATIONS BETWEEN BLOOD PLASMA BIOMARKERS, PET ACCUMULATION, AND CEREBRAL ATROPHY IN ALZHEIMER’S DISEASE

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Aims: We infer spatial signatures of Amyloid PET, Tau PET, and cortical atrophy as correlates of blood plasma biomarkers for Alzheimer’s Disease pathology. We also determine the relative contribution of each imaging modality in relation to these plasma biomarkers, allowing us to uncover potential drivers of these plasma levels.

Methods: Two hundred eighty-seven participants from the Alzheimer’s Disease Neuroimaging Initiative (ADNI) were included. Using partial least squares (PLS) regression, we identified the PET and atrophy spatial signatures of the rates of both retrospective plasma p-tau181 and NfL. By iteratively weighting each imaging modality, we determined to what extent each modality (Amyloid PET, Tau PET, and cortical atrophy) is associated with the plasma biomarkers jointly and separately.

Results: By iteratively weighting (alpha and beta weighting in Figure) each imaging modality, we determined the variance in plasma measures explained by each modality (Aβ PET, tau PET, and cortical atrophy) jointly and separately. The variance observed with the rates of plasma p-tau181 and NfL is largely explained by both rates of tau accumulation and rates of neurodegeneration but to a lesser extent by rates of Aβ accumulation (Figure).

Conclusions: Our current findings support recent literature which suggest that both plasma p-tau181 and NfL levels are well associated with AD-related brain changes. From our iterative PLS regression, we take this one step further and determine what’s driving these plasma levels: Tau accumulation and neurodegeneration. This finding suggests that both these plasma biomarkers may be powerful tools for proactively identifying individuals with certain Tau pathologies that lead to neurodegeneration.
PLASMA LEVELS OF IMMUNOGLOBULIN A AGAINST IAPP ARE ASSOCIATED WITH AD PATHOLOGY IN AN APOE ISOFORM-DEPENDENT MANNER

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Aims: Pancreas-derived Islet amyloid polypeptide (IAPP) co-deposit with amyloid beta in the brain of Type 2 diabetes (T2D) and Alzheimer's disease (AD) patients. The depositions are believed to be related to the amount of circulating plasma IAPP, but it warrants further investigation. Interestingly, T2D patients display elevated levels of IAPP-autoantibodies, confirming the pathological relevance of IAPP. In the current study, we investigate whether AD patients also demonstrate altered levels of IAPP-autoantibodies and whether they are linked to APOE genotype, AD biomarkers, and cognitive decline.

Methods: Plasma levels of IAPP-IgG, IAPP-IgM, IAPP-IgA, total IgA, and IAPP in AD patients and non-demented controls (NCs) were measured by ELISA.

Results: Levels of IAPP-IgG, IAPP-IgM, and IAPP-IgA did not differ between NCs and AD patients, but total IgA was higher in AD patients compared to NCs. Interestingly, levels of IAPP-IgA, but not IAPP-IgG or IAPP-IgM were significantly lower in APOE4 carriers in an allele-dose dependent manner, even though levels of IAPP or total IgA did not differ between APOE4 carriers and APOE4 non-carriers. Finally, IAPP-IgM and IAPP-IgA correlated negatively with amyloid beta 42, hyperphosphorylated tau, and cognitive decline (MMSE) in APOE4 non-carriers, but not in APOE4 carriers.

Conclusions: The relationship between IAPP-IgA, IAPP-IgM, and AD-related variables in APOE4 non-carriers supports the idea that IAPP is implicated in AD pathology. The lack of similar relationship in APOE4 carriers indicates a disturbed IgA response against IAPP in these individuals, which is not linked to the overall IgA production or amount of circulating IAPP.
Aims: This study is aimed to discover blood miRNAs that differentially express in Korean AD patients, evaluate the clinical performance of miRNAs in total plasma or plasma EVs, and investigate the roles of discovered miRNAs in amyloidogenesis.

Methods: Blood from 15 (7 cognitively normal (CN) and 8 AD) out of 262 subjects (59 CN, 105 MCI, 98 AD) and 8 Parkinson's patients was used to discover miRNAs differentially expressed in AD, and we evaluated the clinical performance of the selected miRNAs in plasma and plasma EVs. The effects of discovered miRNAs on Aβ production and expression of their target gene expression were investigated in neuronal culture systems.

Results: Among 18 miRNAs differentially expressed in AD (>2 folds), 3 miRNAs (miR-122-5p, miR-210-3p and miR-590-5p) that were upregulated in AD comparing with both CN and PD were selected. The diagnostic utility of AD or MCI from CN of the selected miRNAs in total plasma or plasma EV was not high. However, the levels of 3 miRNAs in total plasma or plasma EVs of Aβ-PET positive subjects were significantly higher than those of Aβ-PET negatives. Furthermore, the selected miRNAs induced Aβ production through activation of β-cleavage of APP and down-regulated their target genes. Pathway enrichment and PPI network analysis of target genes of the miRNAs supported the roles of the miRNAs in amyloidogenesis.

Conclusions: The diagnostic utilities of circulating mi-ARNAs to discriminate AD from CN were modest. However, the miRNAs were highly expressed in patients with amyloid accumulation, which was supported by in vitro analysis of amyloidogenesis.
BLOOD MICRORNA-150-5P: A NOVEL BLOOD-BASED BIOMARKER FOR ALZHEIMER’S DEMENTIA WITH CORRELATES TO COGNITION, CEREBROSPINAL FLUID AMYLOID-B, AND CEREBRAL ATROPHY

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Aims: The development of blood-based biomarkers that correlate with Alzheimer’s disease (AD) pathology and clinical measures are urgently needed. The aim of this study is to identify and validate a novel blood-based microRNA (miRNA) for dementia of Alzheimer’s disease type (DAT) and to study its correlation to both AD pathology as well as clinical-radiological measures.

Methods: We conducted miRNA-sequencing using peripheral blood mononuclear cells (PBMCs) from a discovery cohort specific for DAT and performed validation in an independent cohort. Correlation analysis evaluated the relationship between miRNA expression and DAT clinical measures, including MMSE and MoCA scores, CSF Aβ1-42 and tau levels, and cerebral atrophy. We also conducted miRNA target gene and pathway analyses.

Results: MiRNA-sequencing identified a distinct miRNAs expression signature differentiating DAT from MCI and HS. Results showed that expression of miR-150-5p was consistent with the miRNA-sequencing data and was further validated. MiR-150-5p expression was upregulated by 73.3% - 76.9% in DAT compared to MCI and HS respectively and discriminated DAT from MCI and HS with high accuracy with AUC 0.86. Higher miR-150-5p correlated with lower cognitive performance, lower CSF Aβ1-42, higher CSF total-tau, and lower default mode and executive control network grey matter volume. Pathway analyses suggest that targets of miR-150-5p to be enriched in the Wnt signaling pathway, including FBXW11 and PDCD4.

Conclusions: miR-150-5p has the potential of being a simple and accurate clinical biomarker for DAT. Future longitudinal studies are needed to determine the clinical utility of miR-150-5p in DAT.
SALIVARY LACTOFERRIN EXPRESSION IN A MOUSE MODEL OF ALZHEIMER’S DISEASE

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Aims: In the last few years, microbial infection and innate immune theories have been proposed as an alternative approach to explaining the etiopathogenesis and origin of Alzheimer's disease (AD). Lactoferrin, one of the main antimicrobial proteins in saliva, is an important modulator of immune response and inflammation and represents an important defensive element by inducing a broad spectrum of antimicrobial effects against microbial infections. We demonstrated that lactoferrin levels in saliva are decreased in prodromal and dementia stages of AD compared with healthy subjects.

Methods: In the present study, we analyzed salivary lactoferrin levels in a mouse model of AD. We collected saliva and submandibular glands from APP/PS1 mice, as well as submandibular gland tissue from AD patients and we analyzed the expression levels of key components of the salivary protein signaling pathway.

Results: We observed robust and early reduction of lactoferrin levels in saliva from 6- and 12-month-old APP/PS1 mice. A significant reduction in M3 receptor levels was found along with decreased acetylcholine (ACh) levels in submandibular glands from APP/PS1 mice. Similarly, a reduction in M3 receptor levels was observed in human submandibular glands from AD patients but in that case, the ACh levels were found to increase.

Conclusions: Our data suggest that the ACh-mediated M3 signaling pathway is impaired in salivary glands in AD, resulting in salivary gland dysfunction and reduced salivary lactoferrin secretion.
PARKINSON’S DISEASE: IDENTIFICATION OF NOVEL SUBSTRATES FOR THE MITOCHONDRIAL KINASE PINK1

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Aims: Mutations in PINK1, a mitochondrial kinase, are linked with familial forms of Parkinson’s Disease (PD). PINK1 coordinates different mitochondrial functions through interaction with multiple substrates. Depending on the overall mitochondrial state, PINK1 can promote the removal of damaged organelles through mitophagy or regulate the mitochondrial function via the phosphorylation of different proteins, including the Complex I subunit NDUFA10. Although several PINK1 substrates have been identified, it appears that none of these can fully restore the PINK1 associated phenotypes in PD disease models. Therefore, the identification of novel PINK1 substrates will help elucidate the mechanisms underlying the PINK1 substrate selection and consequent maintenance of the mitochondrial fitness.

Methods: To achieve this, 2D-DIGE electrophoresis and LC/MS analysis was performed on isolated mitochondria from wildtype (WT) and PINK1 null cells. A phosphoproteome was obtained and following bioinformatic analysis, a list of candidate substrates for PINK1 was generated. To validate these candidates as putative PINK1 substrates, immunoblot analysis and cell-based and in vitro assays were performed.

Results: From the phosphoproteomics screen analysis, five top candidate substrates were selected based on in-silico protein analysis and literature revision. Western Blot analysis revealed distinct expression profiles in WT and PINK1 null cells. Phosphorylation assays will further support the validation of these proteins as PINK1 substrates.

Conclusions: So far, our findings have identified potential novel PINK1 substrates and provided insights on the mechanisms underlying mitochondrial function, particularly in the context of PD, which will ultimately contribute to the development of novel therapeutic strategies for the treatment of the disease.
PLASMA AMYLOID BETA 1-42, TOTAL TAU PROTEIN AND ALPHA-SYNUCLEIN IN PARKINSON’S DISEASE


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Aims: In addition to α-synuclein, amyloid and Tau pathologies were found in patients with Parkinson’s disease (PD). Although results of assaying these proteins in body fluid have been reported, comprehensive studied on the discriminating power between PD and normal control (NC), as well as PD with normal cognition (PD-NC) and PD dementia (PDD) are rare, especially in plasma. In this study, plasma total α-synuclein, amyloid β 1-42 (Aβ1-42) and total Tau in subjects of normal controls (NC), Parkinson’s disease with normal cognition (PD-NC) and Parkinson’s disease dementia (PDD) were assayed in a cohort to explore the roles of these three proteins in PD.

Methods: One hundred and eighty-seven NCs, one hundred and eighteen PD-NC patients and seventy-nine PDD patients were enrolled at five hospitals in Taiwan. Plasma Tau, Aβ1-42 and α-synuclein of each enrolled subject were assayed using IMR.

Results: Plasma Aβ1-42, Tau and α-synuclein significantly increase in PD as compared to NC. Further increases in plasma Tau and α-synuclein were found in PDD dementia as compared to PD-NC. α-synuclein shows the relative strong discriminating power between PD and NC, as well as PDD and PD-NC. Tau shows the relative strong correlation to cognitive decline. In NC, the three proteins are independent on age.

Conclusions: The results suggest that both Tau and α-synuclein play roles in the occurrence of PD and the cognitive impairment in PD. However, Tau level is not associated with the α-synuclein level in PD, which implies that Tau and α-synuclein should be regarded as independent biomarkers in PD.
EVIDENCE OF PLASMA BIOMARKERS INDICATING HIGH RISK OF DEMENTIA IN COGNITIVELY NORMAL SUBJECTS WITH COMORBIDITIES

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Aims: Subjects with comorbidities are at risk for neurodegeneration. There is a lack of a direct relationship between comorbidities and neurodegeneration. In this study, plasma Aβ1-42 and total Tau protein (T-Tau) levels in HRD were assayed. The enrolled HRD included poststroke (PS) subjects, individuals with a family history of AD (ADFH), and patients with diabetes, end-stage renal disease (ESRD) and obstructive sleep apnea (OSA).

Methods: Immunomagnetic reduction (IMR) assays were utilized to assay plasma Aβ1-42 and total tau protein (T-Tau) levels in poststroke (PS, n = 27), family history of Alzheimer’s disease (ADFH, n = 35), diabetes (n = 21), end-stage renal disease (ESRD, n = 41), obstructive sleep apnea (OSA, n = 20), Alzheimer’s disease (AD, n = 65). Thirty-seven healthy controls (HCs) were enrolled.

Results: The measured concentrations of plasma Aβ1-42 were 14.26±1.42, 15.43±1.76, 15.52±1.60, 19.74±2.92, 16.52±0.59, 15.97±0.54 and 20.06±3.09 pg/ml in HC, PS, ADFH, diabetes, ESRD, OSA and AD groups, respectively. The corresponding concentrations of plasma T-Tau were 15.13±3.62, 19.29±8.01, 17.93±6.26, 19.74±2.92, 21.54±2.72, 20.17±2.77 and 41.24±14.64 pg/ml.

Conclusions: The plasma levels of Aβ1-42 and T-Tau in the PS, ADFH, diabetes, ESRD and OSA groups were relatively high compared to HC group but were lower than AD group. This evidence indicates the high risk for dementia in these groups from the perspective of plasma biomarkers.
MACHINE LEARNING FOR THE EARLY DIAGNOSIS OF ALZHEIMER’S DISEASE BASED ON PERIPHERAL INFLAMMATORY IN THE CHILEAN GERO COHORT

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Aims: Our aim is to identify new blood biomarkers to detect the risk to develop AD in the early stages using machine learning based on inflammatory biomarkers and different cognitive domains in the GERO cohort.

Methods: We evaluated subjects derived from the GERO cohort (82 subjective complaints (SC) and 103 mild cognitive impairment (MCI), >70 years), 35 healthy controls (HC) and 32 Alzheimer’s patients (AD). Neuropsychological tests were applied. Plasma inflammatory proteins IL-2, IL-6, IL-10, TNF-α, CRP and SAP were analyzed by Luminex technique. Two machine learning algorithms were developed. There were four diagnosis categories to be predicted: HC, SC, MCI, and AD. The first algorithm was a multiple logistic regression and the second one a classification tree. Both models were checked for multicollinearity to ensure reproducibility. Also, K-fold validation was performed to obtain the accuracy of the algorithms.

Results: In a preliminary stage, the variables that are relevant to classify the patients between the four categories are: scholarship, cytokine IL-6, Free and Cued Selective Reminding Test (FCSRT), Short-term Memory Binding Test (SMBT) and FAS Total. The estimated accuracy for this model is over 0.8.

Conclusions: The combination of different cognitive variables with inflammatory biomarkers allows the construction of a classification model using machine learning methodologies to discriminate between the different stages of cognitive impairment with a high accuracy.
BLOOD BIOMARKERS PREDICTING POOR COGNITION IN HEALTHY AGEING MEN

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Aims: There is a paucity of biomarker studies investigating the preclinical phase preceding the onset of Alzheimer’s disease. The present study aims to identify a panel of blood biomarkers that can predict poor cognition in healthy ageing men.

Methods: The abundance levels of \~270 proteins were measured using a multiple reaction monitoring-based mass spectrometry assay in plasma samples of \~475 male participants. These proteins are commonly found in plasma and known to participate in different physiological processes such as inflammation, oxidative stress, lipid transport, cytoskeleton signalling, coagulation and complement pathway. The sample preparation included removal of high-abundant immunoglobulins, enzymatic digestion using a proteolytic enzyme, desalting of peptides and peptide quantification followed by analysis in a triple-quadrupole instrument. In addition, enzyme-digested peptides were generated in silico, from which only unique peptides were filtered for mass spec monitoring. Stable isotope-labelled internal standards were used for quantification and protein concentrations were determined in fmol/\mu l.

Results: Currently, a statistical analysis is underway that investigates the association between participants’ cognitive scores and protein levels. Furthermore, interactions between cognitive function and comorbidities such as depression and bone loss would also be explored as this may help us tease out any effect comorbidities may have on the relationship between cognition and biomarkers.

Conclusions: We hypothesise that a comprehensive assessment of the blood biochemical changes associated with different degrees of cognitive function in an ageing cohort may lead to the identification of biomarkers for early stages of cognitive decline.
THIMET OLIGOPEPTIDASE IS A POTENTIAL CSF BIOMARKER FOR ALZHEIMER’S DISEASE. A CROSS-PLATFORM VALIDATION STUDY.

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Aims: Using antibody-based proteomics, the neuropetidase Thimet Oligopeptidase (THOP1) was identified as a potential cerebrospinal fluid (CSF) biomarker to discriminate Alzheimer’s Disease (AD) from Lewy body dementia (DLB) and controls. We aimed to develop specific THOP1 immunoassays to facilitate the translation of our biomarker discovery findings into immunoassays for further large scale validation and potential clinical implementation.

Methods: We developed and compared novel immunoassays for CSF THOP1 analysis on automated high-sensitive Ella™ and Simoa™ platforms. The assays were clinically validated in a selection of CSF samples from our previous discovery study (Olink) including 24 cognitively unimpaired controls, 24 AD and 24 DLB patients.

Results: THOP1 levels moderately correlated between proteomics and the novel targeted assays (Olink-Ella: rho=0.702; Olink-Simoa: rho=0.584; Ella-Simoa rho=0.713, all p<0.05). No systematic or proportional differences were detected between assays. Consistent with our discovery findings, THOP1 levels were increased in AD compared to DLB patients (Ella: 1.6-fold and Simoa: 1.7-fold, p<0.05) and controls (Ella: 1.8-fold, p<0.05). THOP1 discriminated AD from DLB with high accuracy (area under curve (AUC): Ella: 0.927 and Simoa: 0.809) or controls (AUC: ELLA: 0.947 and Simoa: 0.839), which was better than CSF amyloid-beta and comparable to Tau forms.

Conclusions: We successfully developed two novel-automated immunoassays replicating increased CSF THOP1 levels in AD, underpinning the potential of CSF THOP1 as a differential diagnostic marker. Our data suggests that the strategy followed, using antibodies in both discovery and validation studies, may facilitate translation of proteomic findings and accelerate the development of body-fluid-based biomarkers.
POSTERS

ACCURATE DISCRIMINATION OF BRAIN AMYLOID STATUS IN THE MULTI-CENTRIC A4 STUDY BY PLASMA Aβ42/AB40 MEASURED WITH A NOVEL HPLC-MS/MS METHOD.

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Aims: To retrospectively explore the ability of Aβ42/Aβ40 plasma ratio, as measured with a novel antibody-free HPLC-MS/MS method (ABtest-MS, Araclon Biotech), to predict the brain amyloid status in a subset of cognitively unimpaired individuals (CU) from the screening visit of the A4 study.

Methods: Aβ40 and Aβ42 plasma levels from 731 CU participants were quantitated with ABtest-MS. Plasma samples were obtained in 59 recruitment sites across USA, Canada and Australia. Logistic regression modeling and ROC curve analysis were carried out to assess the ability of Aβ42/Aβ40, as measured with ABtest-MS, to predict amyloid brain status. Amyloid positivity for 18F-Florbetapir PET brain imaging was established for standardized uptake value ratios (SUVR) ≥ 1.15.

Results: Plasma Aβ42/Aβ40 values were significantly lower in the Aβ-PET positive than in the Aβ-PET negative group (p=3.7·10^{-35}). Log(Aβ42/Aβ40) showed a significant negative correlation with 18F-Florbetapir-PET SUVR values (rho=-0.440; p=2.9·10^{-36}). Plasma Aβ42/Aβ40 discriminated Aβ-PET positivity with an AUC [95% CI] of 0.78 [0.75-0.86]. After inclusion of recruitment site as a covariate in the regression model, AUC increased up to 0.86 [0.83-0.89]. Noteworhily, a full regression model including ratio, recruitment site, age, gender and number of APOE4 alleles, yielded an AUC of 0.88 [0.86-0.91] (accuracy 81.3%).

Conclusions: ABtest-MS accurately identifies amyloid brain deposition in this subset of CU individuals from the A4 study. These results suggest that despite the standardization of the pre-analytical variables, the effect of the recruitment site is not negligible. Therefore, an extensively validated, robust and centralized sample analysis would be highly desirable to minimize this additional variability.
POSTERS

PHOSPHORYLATED-TAU 181 IN PERIPHERAL BLOOD AND PROGRESSION TO AD DEMENTIA IN MCI PATIENTS

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**Aims:** Blood-based biomarkers are promising biomarkers for diagnosis of Alzheimer's disease (AD) at prodromal stages (Mild Cognitive Impairment – MCI). Assays for the quantification of phosphorylated tau 181 (p-tau181) in blood have been recently developed and showed increased concentrations in the Alzheimer's clinical continuum. In this work we evaluated whether peripheral p-tau181 could predict progression to AD dementia in MCI patients.

**Methods:** A group of 96 MCI patients followed at the Dementia clinic of Centro Hospitalar Universitário de Coimbra (CHUC) was included. Data regarding baseline neuropsychological evaluation, cerebrospinal fluid (CSF) levels of Ab42, Ab40, t-tau and p-tau181 was available. Baseline p-tau181 levels were determined in stored serum samples by Single Molecule Array (SiMoA). Progression from MCI to AD dementia was assessed at follow-up (mean 5.1±3.1 years).

**Results:** Baseline serum p-tau 181 levels showed a moderate correlation with p-tau181 in CSF (r=0.550; p<0.001), and were significantly increased in patients classified within the AD-continuum according to their CSF biomarker profile (p<0.001). Serum p-tau181 was associated with decreased global cognition at baseline (p<0.05) and was significantly increased in patients that progressed to AD at follow-up (p<0.001). Serum p-tau181 demonstrated a diagnostic accuracy to identify progression to AD dementia similar to the CSF markers (AUC=76.6% for serum p-tau181; p>0.05 for comparison with all markers in CSF) and higher baseline concentrations of serum p-tau181 were an independent predictor of future AD dementia (HR=4.1; 95%CI=2.0-8.2).

**Conclusions:** Our results show that p-tau181 measurements in peripheral blood have potential as a non-invasive prognostic tool for AD dementia in MCI patients.
POSTERS

RHBDF2 AND HAND2 DNA METHYLATION AS POTENTIAL BIOMARKERS OF NEURODEGENERATION IN PERIPHERAL BLOOD.

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Aims: CSF biomarkers are a useful tool in the diagnosis of Alzheimer’s Disease (AD) but peripheral blood is lately emerging as a non-invasive source of biomarkers. Epigenetic biomarkers, as DNA methylation, are stable, reproducible and easily quantifiable. Thus, we aimed to search for blood-based epigenetic biomarkers for AD diagnosis.

Methods: We analyzed the correlation between CSF biomarkers levels and DNA methylation levels for differentially methylated genes in DNA of peripheral blood leukocytes of 26 patients with probable AD dementia based on NIA-AA criteria. We assessed DNA methylation levels for 18 candidate genes by bisulfite pyrosequencing. All participants were genotyped for APOE. Aβ42, Aβ40, Aβ42/Aβ40, tTau and pTau181 protein levels were measured by automated chemiluminescent enzyme immunoassay (CLEIA) and NfL CSF by ELISA.

Results: Negative correlation was found between RHBDF2 DNA methylation levels and tTau \( r = 0.49; p \text{-value}<0.01 \) and pTau181 \( r = 0.45; p \text{-value}<0.05 \) protein levels. HAND2 DNA methylation negatively correlated with tTau \( r = 0.48; p \text{-value}<0.05 \), pTau181 \( r = 0.38; p \text{-value}<0.05 \) and Aβ40 \( r =-0.38; p \text{-value}<0.05 \). No correlation was found between Aβ42/Aβ40 ratio and DNA methylation levels. We also conducted subgroup analyses stratified by APOE4 to investigate the effects of APOE genotype on the association between DNA methylation and AD CSF biomarkers. RHBDF2/tTau correlation remained statistically significant in E4 allele non-carrier group \( r =-0.57; p \text{-value}<0.05 \) and HAND2/tTau in E4 allele carrier group \( r = -0.53; p \text{-value}<0.05 \), respectively.
**Conclusions:** These results suggest that quantification of RHBDF2 and HAND2 methylation levels may be potentially useful to identify neurodegeneration. These genes emerge as promising epigenetic biomarkers which are worth exploring in larger cohorts to assess their diagnostic value.
CROSS-SITE VALIDATION OF CSF PLACENTAL GROWTH FACTOR (PLGF) AS A FLUID BIOMARKER FOR CEREBRAL SMALL VESSEL DISEASE: A MARKVCID CONSORTIUM STUDY.

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Aims: Placental growth factor (PIGF) is a member of the VEGF family of angiogenic mediators, and we identified a relationship between CSF PIGF and volume of white matter hyperintensities (WMH). As part of the MarkVCID consortium, we sought to validate CSF PIGF as a biomarker for cerebral small vessel disease (cSVD) across consortium sites.

Methods: CSF PIGF analysis by Quanterix Simoa HD-X.

Results: Validating the reliability of the assay across sites, three aliquots of 20 CSF samples (10 sourced from The University of Kentucky (UKY), and 10 from The University of New Mexico (UNM)) were blinded and individual aliquots provided to UKY, UNM, and The University of Texas Health Science Center at San Antonio (UTHSCSA) sites. Each site measured PIGF using Quanterix Simoa. We found an inter-class correlation coefficient (ICC) between all sites of 0.94, indicating good reproducibility across the sites. Validating the utility of CSF PIGF as a useful biomarker of cSVD, each site measured 40 CSF samples and determined the association of CSF PIGF with both cognition and WMH. At UKY, regression analysis found a modest association of higher CSF PIGF with greater WMH volume but stronger inverse associations with two different verbal fluency tests. At UNM, only modest associations were identified of CSF PIGF with WMH and TRAILS-A. At UTHSCSA (combining UTHSCSA and JHU CSF samples), CSF PIGF showed a significant association with TRAILS-A test.

Conclusions: Overall, our data suggest CSF PIGF is a candidate biomarker for cSVD, but more studies with a larger sample are necessary to further validate this finding.
POSTERS

CEREBROSPINAL FLUID BIOMARKER PANEL FOR SYNAPTIC DYSFUNCTION IN PARKINSONISM

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Aims: Synaptic dysfunction and degeneration are early characteristics of neurodegenerative diseases such as Parkinson’s disease (PD). Consequently, biomarkers capable of measuring these processes could potentially serve as early diagnostic and prognostic tools for PD and related disorders. We have successfully developed a novel cerebrospinal fluid (CSF) panel to concurrently quantitate 17 synaptic proteins, including SNARE proteins, synucleins, neuronal pentraxins, and neurogranin. In this study, we aimed to study the synaptic proteins in the Parkinson spectrum, including PD, corticobasal degeneration (CBD), progressive nuclear palsy (PSP), and multiple system atrophy (MSA).

Methods: One hundred µL CSF samples were prepared in a three-step sample preparation comprising of stable isotope-labeled peptide standards addition, tryptic digestion, and purification by solid-phase extraction. Micro-high-performance liquid chromatography-mass-spectrometry was used for quantification. A cross-sectional study including controls (n=48), PD (n=51), CBD (n=11), PSP (n=22), and MSA (n=31) was performed.

Results: The most noteworthy results were found for the pentraxins and neurogranin, where all pentraxins (1, 2, and the receptor) were decreased in PD, PSP, and MSA compared to controls. Similar results were found for neurogranin. No differences were found for any proteins for CBD.

Conclusions: We have successfully implemented a novel CSF panel for synaptic dysfunction containing 17 biomarkers. Our results indicate that several of the panel proteins show potential to be used as synaptic degeneration or dysfunction biomarkers not only in AD but also in parkinsonian disorders, such as PD, PSP, and MSA.
PLASMA P-TAU181 AND P-TAU231 REFLECT CEREBROSPINAL FLUID BIOMARKERS OF ALZHEIMER’S DISEASE

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Aims: Phosphorylated tau 181 and 231 (p-tau181 and p-tau231) have been recently identified as potential peripheral markers of Alzheimer’s disease (AD) pathology. Still, the relationship between plasma and cerebrospinal fluid AD biomarkers need further investigations to be implemented in clinical practice.

Methods: Consecutive subjects with cognitive impairment underwent an extensive neurological, neuropsychological and behavioral assessment and standard CSF analyses for AD biomarkers. Plasma and CSF p-tau181, p-tau231 were analyzed using SIMOA Platform (Quanterix). The between-groups differences in plasma and CSF biomarkers were evaluated using non-parametric Kruskall-Wallis analyses and discriminative analyses; the relationship between plasma/CSF biomarkers was evaluated using correlation analyses in whole group and AD patients only.

Results: seventy-four subjects with CSF and plasma assessment entered the study, namely 58 patients with cognitive impairment classified as AD (n=43) and non-AD (n=16) and 16 healthy controls (HC). CSF and plasma p-tau181 and p-tau231 levels increased in AD patients and positively correlated with CSF p-tau/Aβ42 ratio, T-tau and p-tau markers. Both markers negatively correlated with Aβ42 and cognitive function at the time of assessment. Plasma p-tau181 exhibited slightly higher discriminative accuracy for CSF p-tau/Aβ42 compared to p-tau181 (AUC 0.87 vs 0.81, respectively).

Conclusions: CSF and plasma p-tau181 and p-tau231 exhibited high accuracy and strong correlation with CSF AD pathology markers and disease severity measures. Larger studies are warranted to confirm and extend these findings in early stages of the diseases.
A MODEL OF THE TURNOVER OF SOLUBLE AMYLOID PRECURSOR PROTEIN-BETA AND CHANGES IN TURNOVER RATES IN ALZHEIMER’S DISEASE

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Aims: We hypothesize that a subgroup of the AD and non-demented Amyloid+ populations overproduce Aβ because of increased BACE1 activity. Our objective is to measure CSF sAPPβ and sAPPα turnover rates, as surrogate markers of BACE1 activity, to determine if, and by how much, BACE1 activity is increased.

Methods: Using stable isotope labeling kinetics/immunoprecipitation/liquid chromatography-tandem mass spectrometry methods, we quantified sAPPβ and sAPPα in CSF from human Amyloid+ (AD) and Amyloid- (control) subjects who had undergone [U-13C6]-leucine labeling and hourly CSF collection. The fraction of metabolite derived from de novo synthesis was measured by calculating normalized metabolites’ hourly mole fraction labeled (MFL), over 36 hours. A model of sAPP kinetics was derived, which included these subjects’ historical Aβ measurements, to study parameters of fractional turnover rates (FTR) within the whole system.

Results: Our initial model of this incomplete cohort indicates sAPPα and sAPPβ turn over slower than the Aβ peptides, and sAPPα turns over a little faster than sAPPβ in most subjects (more pronounced in the setting of amyloidosis). There is almost a significant amyloid effect on both the single compartment FTR and the whole system FTR for both peptides, and not quite a trend for faster whole system FTR for sAPPα than sAPPβ.

Conclusions: Our initial model allows extraction of kinetic parameters that describe well the shape of APP peptide SILK curves. It isn’t a physiological model and was intended to see if there was anything interesting to show. Development of more physiological models are ongoing with data collection from the larger cohort.
A SYSTEMATIC REVIEW AND META-ANALYSIS OF CEREBROSPINAL FLUID MARKERS OF THE TRANSITION FROM MILD COGNITIVE IMPAIRMENT TO ALZHEIMER'S DISEASE

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Aims: To identify individuals with increased probability of developing Alzheimer’s disease (AD), prior studies have investigated amyloid-beta (Aβ) and tau in cerebrospinal fluid (CSF). This study systematically evaluated reported candidate biochemical biomarkers, to identify their effectiveness in predicting the transition from Mild Cognitive Impairment (MCI) to AD.

Methods: A systematic review of the literature was performed for studies reporting immunologically-based (xMAP or ELISA) measures of CSF Aβ and tau in longitudinal clinical studies. Of 1137 screened publications, 25 met the inclusion criteria.

Results: CSF tau and Aβ levels were differentiated by patient outcome. Aβ concentration was measured at two or more time-points in 24 studies. T-tau levels were reported in 21 studies; P-tau levels in 18. Absolute levels were evaluated, as well as tau/Aβ ratios from a subset of eight papers. With xMAP and ELISA, Aβ42 levels were significantly lower in MCI patients who progressed to a diagnosis of AD compared to those who did not, or to healthy controls. The opposite was observed for P-tau and T-tau, where MCI patients progressing to AD had the highest levels. Non-progressing MCI and healthy control groups had similar levels of CSF tau. The P-tau/Aβ42 ratio gave the most reliable indication of whether a patient would transition from MCI to AD.

Conclusions: While the roles of amyloid-beta and tau remain controversial, their suggested value as clinical predictors of MCI to AD conversion was supported by these findings. Opportunities to strengthen future study design for CSF evaluation of Aβ and tau were identified, with the follow-up interval in longitudinal evaluations being particularly critical.
POSTERS

DECREASE OF IMMUNE AND INFLAMMATORY BIOMARKERS IN THE BDNF DECREASED MIDDLE-AGED WOMEN PARTICIPATED IN SHORT-TERM BEACH FITNESS RETREAT PROGRAM

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Aims: The level of brain-derived neurotrophic factor (BDNF) decreases with neurodegeneration, and this process correlates with AD stages. BDNF level is also affected by exercise. Several studies have reported that the level of BDNF increased after exercise. However, the decrease of BDNF has been reported in the group who joined exercise retreat program for 3 months. The objective of this study is to evaluate the change of immune/inflammatory biomarkers with the level of BDNF by short-term exercise retreat program in Wando, Korea for normal middle-aged women.

Methods: The total of thirty-seven women who are 50s and 60s was recruited. The study participants performed nordic walking and beach yoga every day for five days. Blood was collected twice before and after the program.

Results: We compared the immune/inflammatory markers between BDNF decrease (BDG) and increase group (BIG). BDG was older than BIG and showed significant decrease of markers including pancreatic polypeptide Y, transforming growth factor-β, monocyte chemoattractant protein-1 and tumor necrosis factor-α comparing to BIG. However, the change of amyloid beta between the BDG and BIG showed no significant differences.

Table. Changes of immune/inflammatory markers and amyloid beta between BDNF decrease and increase groups for normal middle-aged women participated in short-term beach fitness retreat program

<table>
<thead>
<tr>
<th>Marker</th>
<th>BDNF decrease group (n=17)</th>
<th>BDNF increase group (n=20)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic Polypeptide Y (PPY)</td>
<td>-93.11±100</td>
<td>-10.51±95</td>
<td>0.015*</td>
</tr>
<tr>
<td>Transforming Growth Factor-β (TGF-β)</td>
<td>-11.04±6.48</td>
<td>-2.61±6.92</td>
<td>0.001**</td>
</tr>
<tr>
<td>Monocyte Chemoattractant Protein-1 (MCP-1)</td>
<td>-79.89±66</td>
<td>-7.90±105</td>
<td>0.019*</td>
</tr>
<tr>
<td>Tumor Necrosis Factor-α (TNF-α)</td>
<td>-0.11±0.15</td>
<td>0.03±0.12</td>
<td>0.003*</td>
</tr>
<tr>
<td>Amyloid β (Aβ)</td>
<td>&lt;0.01±0.32</td>
<td>0.02±0.25</td>
<td>0.845</td>
</tr>
</tbody>
</table>

Statistical significance was assessed using independent sample t-test.
Conclusions: Fitness program for BDG showed significant decrease of the markers, but not for BIG. And BDG is older than BIG. These results suggested effects of short-term fitness retreat program to brain health may different among middle-aged women according to ages and other factors. Further studies are needed to find the associations between BDNF and immune/inflammatory markers. And these findings should be tested in larger population.
Aims: For the prevention of dementia, noninvasive screening methods which detect the signs of disease progression and/or evaluate the risk of dementia before clinical symptom occurs are needed. Here, we analyzed plasma biomarkers related to Aβ clearance and neuroinflammation by LC-MS/MS MRM assay, and established LC-MS blood test for MCI and AD to explore accurate and practical assessment of cognitive impairment at an early stage.

Methods: We constructed a robust and reproducible LC-MS/MS system equipped with MRM to quantify 45 major plasma proteins with relatively high amounts using isotope-labeled synthetic peptide, and these plasma protein levels from 192 cases (NDC: 58, MCI: 71, AD: 63) were determined. Multinomial regression and ROC analyses were performed to identify the combination of the proteins that discriminated NDC vs. MCI and/or AD. Furthermore, more than 100 cases were evaluated in the prospective study.

Results: We found eight interpretable protein biomarker candidates in plasma for MCI and AD, which were related to innate immunity, coagulation pathways, lipid metabolism, and nutrition, and some of them are involved in LRP1-mediated Aβ clearance and neuroinflammation. The optimal combination of these proteins and the coefficients of the logistic models revealed different clinical potential between male and female. In the model discriminating NDC vs. cognitive impairment, the AUC values of the ROC analysis were 0.82 and 0.72 for male and female, respectively. Furthermore, the composite scores were correlated with the severity of cognitive decline.

Conclusions: LC-MS-based plasma protein panel interpretable for pathophysiology of AD is useful for evaluate the risk of the disease progression.
Aims: Amyloid pathology-confirmed diagnosis of Alzheimer’s Disease (AD) has been an increasing area of focus. Assistance of diagnosis using amyloid PET imaging or cerebrospinal fluid biomarkers has been challenging to implement in clinical care due to cost, availability, and/or invasiveness of sampling. These hurdles can be circumvented by the assessment of biomarkers in the more easily accessible plasma. The aim of this study was to evaluate a plasma pTau biomarker as a tool for predicting amyloid pathology.

Methods: The sample set consisted of 20 AD cases, 25 Mild Cognitively Impaired (MCI) subjects, and 20 controls (HC). Per amyloid PET, the cohort included 26 positives (including all ADs) and 39 negatives (including all HC). CSF and plasma biomarker analysis were done on the fully automated LUMIPULSE G platform (Fujirebio) using four commercial CSF assays for Aβ1-40, Aβ1-42, tTau, and pTau181 and a prototype assay for plasma pTau181.

Results: The Amyloid pathology phenotyping using CSF biomarker ratios aligned closely with PET imaging with overall percentage agreement above 90% for all ratios evaluated. The average plasma pTau181 concentration between Controls (2.31 pg/mL) and ADs (4.15 pg/mL) differed significantly. The amyloid pathology discrimination capability of the prototype plasma pTau181 assay for this cohort, as determined by ROC analysis, was found to be good (AUC > 0.7).

Conclusions: The high concordance between Lumipulse AD CSF biomarkers and amyloid PET imaging was confirmed in this cohort. The Lumipulse pTau181 plasma prototype showed significant promise for use as an AD diagnostic / monitoring tool.
ASSESSMENT OF POTENTIAL SCREENING PROCEDURE IN EARLY AND DIFFERENTIAL ALZHEIMER DISEASE DIAGNOSIS

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Aims: Alzheimer disease (AD) is the leading cause of dementia in elderly population. Current diagnosis is based on invasive and expensive techniques, so there is a growing need to look for other possible tests, as well as to carry out a clinical validation. Studies from literature showed potential diagnosis models developed including some AD risk factors (age, gender, ApoE-E4 genotype), and other variables (biomarkers levels, neuroimaging). Specifically, a recent model was performed from lipid peroxidation compounds in plasma samples to identify patients with early AD. The aim of this study is to validate our previous model for early AD diagnosis in plasma samples based on lipid peroxidation biomarkers and to improve this model including other AD risk factors.

Methods: Plasma samples from participants classified into AD (n=61), non-AD (n=17) and healthy (n=44) were analysed. In fact, lipid peroxidation compounds were determined by liquid chromatography and mass spectrometry. Then, a previously developed diagnosis model was clinically validated, evaluating some diagnosis indexes.

Results: The validation of the preliminary diagnosis model showed satisfactory diagnosis indexes (accuracy 77%, sensitivity 89%, specificity 61%, diagnostic odds ratio 12.5, positive predictive value 76%). Then, a useful screening tool including the ApoE genotype was developed, identifying patients with higher risk to develop AD and improving the diagnosis indexes (accuracy 82%, sensitivity 81%, specificity 85%, diagnostic odds ratio 23.2, positive predictive value 90.5%).

Conclusions: A new screening approach improved the early, minimally invasive and differential AD diagnosis in general population.
MOLECULES EXPRESSING IN BRAIN SENESCENT GLIAL CELLS SPECIFICALLY DIFFERENTIATE ALZHEIMER’S DISEASE FROM NORMAL AGING AND NON-ALZHEIMER’S DEMENTIA

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Aims: Aging is a major risk factor for AD. Cellular senescence occurs physiologically as aging. In the present study, we aimed to investigate how the road of healthy aging is off to the degenerative path and whether there are biomarkers to differentiate healthy aging and neurodegenerative process.

Methods: Entire brain senescence was assessed in 29 postmortem brains, and CSF biomarkers related to senescent cells were investigated with China Aging and Neurodegenerative Disease Initiative (CANDI) cohort.

Results: Senescent cells are present in postmortem brains from healthy elderly individuals and further accumulate in the AD brains. Interestingly, senescent cells are predominantly glial cells, including astrocytes and oligodendrocyte lineage cells. Moreover, typical pro-inflammatory molecules, which are upregulated during aging, were significantly elevated in the AD brain and most of them were labeled by senescent cells. Furthermore, these molecules in the CSF are associated with the entire brain senescence. Most of the aging and inflammatory molecules were associated with amyloid-β or tau pathology. Notably, CSF YKL-40 and MIF were markedly elevated in older individuals, but significant changes of HGF, MIF, and TSP2 were elevated in older individuals with AD pathology. We also reveal that YKL-40, TSP2, and SerpinA3 are useful biomarkers for discriminating AD from CN and non-AD patients.

Conclusions: We demonstrate the corresponding CSF biomarkers of the abnormal accumulation of senescent glial cells in AD brains and reveal that the CSF aging molecule panel related to senescent glial cells are potential biomarkers to track the senescence status of glial cells.
Aims: The amyloid β (Aβ) peptide can exist in many structural isoforms, which can be detected by a variety of different antibodies. While ELISA and SPR assays can only provide information on affinity, the IRS technology can additionally resolve the secondary structure of the bound Aβ peptides. Here we show a reversible label-free immuno-infrared based platform to detect changes in the secondary structure distribution of the structural spectrum of Aβ-isoforms.

Methods: This assay combines the secondary-structure-specific infrared spectroscopy, in a flow system based attenuated total reflection (ATR) set-up, with methods of immuno-detection by protein-tag immobilized FAB-fragments. The surface is based on the interaction between a cognate bacterial protein pair and is reversible by the use of high salt buffers.

Results: We have developed a screening platform that can perform multiple cycles of FAB fragment binding. We can characterize the structural properties of artificial and naturally occurring Aβ isoforms bound to AD-relevant antibody fragments by secondary structure analysis of the amide I absorption.

Conclusions: The newly developed screening platform allows a large number of measurements to be performed in a resource-efficient manner without the time-consuming procedure of chemical surface preparation in each cycle. This fully automated assay can be used as a tool e.g., for characterization of AD-related peptides and analysis of their structural properties.
**Aims:** Amyloid pathology is a key hallmark of Alzheimer's disease (AD). With the development of various disease modifying therapies, we consider that the demand for simple testing methods such as blood-based biomarkers will increase. Recently, we developed fully automated immunoassays to measure plasma Aβ1-40 and Aβ1-42. Here, we evaluated the detailed analytical performance of these reagents.

**Methods:** The sensitivity, precision, cross-reactivity, and interference from endogenous substances were evaluated. To verify the immunoassays specificity, we assessed the correlation with immunoprecipitation mass spectrometry (IP-MS) assays using plasma samples.

**Results:** The limits of quantification were 2.46 pg/mL and 0.16 pg/mL for Aβ1-40 and Aβ1-42, respectively. The repeatability (within-run) coefficients of variation (CVs) were less than 3.7% (Aβ1-40) and 2.0% (Aβ1-42). We found that the intermediate precision (within-laboratory) CVs were less than 4.6% (Aβ1-40) and 5.3% (Aβ1-42). The cross-reactivity with various lengths of Aβ peptide was less than 0.5% and the interference from blood components was less than 10% for both assays. Finally, we showed that there were significant correlations between our assays and IP-MS assays with correlation coefficients of Pearson's r = 0.91 (Aβ1-40) and 0.82 (Aβ1-42).

**Conclusions:** We have developed robust, sensitive, and highly specific immunoassays for Aβ1-40 and Aβ1-42 without the interference from blood components and cross-react from other Aβ peptides. Our fully automated immunoassays are feasible to use for high-throughput testing, suggesting that our assays may have a potential for use in clinical practice.
AN INFRARED-IMMUNO-SENSOR DETECTING THE AMYLOID-BETA-MISFOLDING IN BODY FLUIDS FOR PRECLINICAL TO SEVERE ALZHEIMER’S DISEASE DIAGNOSIS

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Aims: Alzheimer's Disease (AD) is accompanied by misfolding of the amyloid β (Aβ) peptide into beta-sheet enriched structures with high neurotoxicity. We have developed a diagnostic infrared-immuno-sensor (IRIS) that monitors the secondary structure distribution of Aβ as an early detectable biomarker before the clinical manifestation and progress of AD.

Methods: The infrared-immuno-sensor combines the secondary-structure-specific infrared spectroscopy, in a flow system-based attenuated total reflection (ATR) set up, with methods of immuno-detection by surface-immobilized antibodies. The antibodies enable us to capture the disease-associated proteins (e.g. Aβ) from complex media such as blood plasma or cerebrospinal fluid (CSF) and monitor the secondary structure distribution by ATR-IR-spectroscopy. The methodical read-out is the amide I maximum frequency (1620-1660 cm⁻¹) of the total-Aβ, which depends on its secondary structure distribution in bodyfluids. The lower the amide I frequency, the higher is the content of misfolded Aβ species in the total-Aβ-fraction.

Results: We validated the diagnostic performance of the infrared-immuno-sensor in four independent clinical studies (Essen, Amsterdam, BioFINDER, ESTHER) focusing on preclinical, prodromal, and mild to moderate AD disease stages. In combination with structure-based Tau analyses in CSF we could observe a sensitivity of 87% and a specificity of 97%.

Conclusions: Our results demonstrate a highly specific and sensitive immuno-assay capable of tracking structural changes of Aβ before clinical manifestation of AD due to its unique combination with ATR-IR-spectroscopy. Further improvements regarding throughput and surface chemistry optimization are in progress and will empower our technique further.
PEPTIDE AGGREGATES ON RED BLOOD CELLS MEASURED BY ATOMIC FORCE MICROSCOPY AS PHYSICAL BIOMARKERS OF ALZHEIMER’S DISEASE PATHOLOGY

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Aims: To resolve and quantify peptide aggregation on red blood cells (RBCs) with atomic force microscopy and evaluate whether protein aggregates on RBCs are a sensitive biomarker of Alzheimer's disease pathology.

Methods: Here, using a benchtop atomic force microscope (AFM) we profiled protein aggregates adsorbed on red blood cells (RBCs) from patients with cognitive complaints at a memory clinic. We present the results of the first 50 patients (19 patients with AD dementia, 3 with primary progressive aphasia, 2 with vascular dementia, 2 with Lewy Body dementia, 4 with unspecified dementia, 12 patients with MCI and 8 patients with subjective cognitive decline), and 16 healthy individuals.

Results: The AFM images with sub-2nm spatial resolution revealed stark differences in size, shape and distribution of protein aggregates, which was observed to depend on patient age and stage of neurocognitive disorder. Interestingly, crystallographic domains composed of aligned single-fibrils were exclusively detected on RBCs for AD patients aged between 80-89 years. The prevalence of fibrillar aggregates was negatively correlated with the CSF Aβ-42/40 ratio and was observed to be significantly higher in the amyloid positive patient category. Using a cut-off of ≥ 40% prevalence of fibrillar aggregates on RBC the CSF-amyloid status could be classified with 88% accuracy (sensitivity 100%, specificity 73%).

Conclusions: Peptide aggregates on RBCs can be reliably visualized and quantified with AFM. Although our data currently lacks chemical information, the morphological insights gained on the spatial organisation of peptide aggregates on RBCs could still represent a novel screening biomarker of AD pathophysiology.
DEVELOPMENT OF AN ULTRASENSITIVE NEUROFILAMENT LIGHT CHAIN (NFL) IMMUNOASSAY FOR THE SMCxPRO™ ASSAY PLATFORM

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Aims: The recent advent of ultrasensitive assay technologies has enabled the measurement of low-abundance blood-based biomarkers of neurodegenerative conditions, thereby addressing the need for invasive nature of cerebrospinal fluid (CSF) collection, allowing for large scale sample acquisition and screening in research, and empowering studies into the transition between health and disease. Utilizing the SMCxPRO™ ultrasensitive immunoassay platform, we have developed an assay for the detection of neurofilament light chain (NFL) in human serum, plasma and CSF samples across healthy and diseased states. NFL is a valuable neurodegenerative biomarker for accessing progression and treatment of Alzheimer’s Disease (AD), Multiple Sclerosis (MS) and Huntington’s Disease, and is under investigation as a potential biomarker for several other neurological disorders.

Methods: Rigorous assay development procedures were undertaken in an effort to produce an NFL immunoassay capable of accurately and reproducibly detecting NFL in human serum, plasma, and CSF samples.

Results: A robust kit for consistent and reliable NFL measurement at sub-pg/mL concentrations has been developed and characterized using Single Molecule Counting (SMC™) ultrasensitive immunoassay technology. The developed assay demonstrates excellent performance characteristics for spike/recovery values and dilutional linearity (90-110% for all three matrices). NFL was detected in all sample types tested. Increased NFL sample levels in AD and MS samples as compared to normal serum, plasma and CSF samples was also observed.

Conclusions: Our SMC™ NFL immunoassay kit provides a valuable tool for research in the areas of neurodegenerative disease and neurological injury.
COMBINATION OF AMYLOID-BETA STRUCTURE AND GFAP LEVELS IN PLASMA PREDICTS ALZHEIMER’S DISEASE UP TO 17 YEARS BEFORE CLINICAL DIAGNOSIS

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Aims: Plasma biomarkers for Alzheimer’s disease (AD) have emerged as crucial risk assessment tools. Since a disease modifying therapy is available, determination of the best therapeutic window based on biomarkers is urgently needed. Therefore, the aim of this study was to characterize participants in a community-based cohort study followed over 17 years biochemically based on the plasma biomarkers, amyloid-beta (Abeta) misfolding, phosphorylated tau (p-tau181), glial fibrillary acidic protein (GFAP), and neurofilament light (NfL). Disease prediction accuracy of AD diagnosis throughout follow-up based upon combinations of biomarkers measured at baseline was examined.

Methods: Abeta misfolding in plasma taken at baseline was determined by an immuno-infrared sensor. P-tau181, GFAP, and NfL concentrations were measured by Simoa technology. 68 participants received an AD diagnosis within 17 years of follow-up and 240 controls without dementia diagnosis were included.

Results: Participants who received an AD diagnosis within 17 years had significantly higher Abeta misfolding at baseline compared to controls. Moreover, significantly higher plasma levels of p-tau181, GFAP and NfL at baseline were seen in AD cases compared to controls. Abeta misfolding was the best performing biomarker and the combination with GFAP levels led to increased disease prediction accuracy, where p-tau181 and NfL did not improve disease prediction accuracy significantly.

Conclusions: Abeta misfolding and GFAP as a blood-based biomarker panel exhibited high disease prediction accuracy even many years before AD diagnosis (17years), which suggests potential as an AD prescreening tool for older adults.
Aims: We have developed an immuno-Infrared sensor that determines the secondary structure distribution of Aβ in blood plasma as structure based biomarker. The biomarker was validated in three independent clinical studies and correlate to established CSF biomarkers and PET scanning. Especially very early preclinical symptom-free Alzheimer’s disease (AD) can be identified. However, up to now Fourier-transform infrared (FTIR) spectroscopy was used. Here we present a miniaturized and user-friendly IR Immuno sensor (iRIS) based on the newest generation of quantum cascade lasers.

Methods: iRIS uses a QCL-light source instead of a globar and room temperature detectors in contrast to liquid nitrogen cooled MCT detectors used in FTIR instruments. The secondary structure distribution of Aβ is measured by a surface functionalized ATR.crystal with Aβ–specific antibodies and an automated flow-through system.

Results: The QCL-based system provides similar signal to noise ratio as compared to FTIR. The amide I bands has the same shape as compared to FTIR measurements. Overall, we show that the iRIS system can be used to perform measurements on protein monolayers for secondary structure determination. It can identify Alzheimers in CSF and plasma in a symptom-free stage.

Conclusions: Compared to the FTIR the iRIS-system is about 12 times smaller, the size is 25x25x10 cm³. It is much easier to handle, and needs neither liquid nitrogen nor vacuum. IRIS is now suitable for clinical application.
A COMPOSITE BLOOD-BASED BIOMARKER FOR PROGRESSION OF COGNITIVE IMPAIRMENT FROM THE PRECLINICAL STAGES TO ALZHEIMER’S DISEASE

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Aims: Blood biomarkers monitoring the disease progression in Alzheimer's disease (AD) are important for early diagnosis and intervention. To verify usefulness of blood biomarkers in monitoring the disease progression, we analyzed plasma levels of Brain-derived neurotrophic factor (BDNF), Aβ40/42 and the Aβ sequester proteins in subjective cognitive impairment (SCD) as the preclinical stages, MCI, and AD.

Methods: In a multicenter clinical study, a total of 164 subjects consisting of 30 AD, 58 MCI, and 76 SCD and 30 Non demented control (NDC) were included in this study, and plasma levels of BDNF, Aβ42, Aβ40, and proteins involved in Aβ sequestration (Triple marker as a composite of apoA1, C3, TTR) were analyzed.

Results: In receiver operating characteristic (ROC) analysis, the triple marker had AUC values of 0.63, 0.71 and 0.82 in NDC vs. SCD, MCI, and AD, respectively. By combining the triple marker and Aβ42 / 40, the AUC values were 0.77, 0.85 and 0.91 in NDC vs. SCD, MCI, and AD, respectively. BDNF was significantly reduced in AD compared to SCD, MCI and NDC. When BDNF was combined with the triple marker plus Aβ42 / 40, high AUC value of 0.96 was obtained in SCD vs. AD.

Conclusions: BDNF is involved in survival of nerve cells and has reduced plasma levels in AD, however, plasma BDNF has limited clinical efficacy as a biomarker for SCD and MCI. Combination of these blood biomarkers may be useful to detect early stages of dementia and monitor the progression of the disease.
Aims: Aβ peptides play a key role in the diagnosis of Alzheimer's disease (AD), as these marker peptides already show abnormal properties at an early stage of the disease, including endogenous concentration distribution and structural conformation. We have previously established an immuno-infrared sensor based on ATR-FTIR spectroscopy to determine the secondary structure distribution of Aβ peptides in plasma and CSF revealing specific Aβ peptide secondary structure at an early AD stage. In this work, we present the combination of immuno-infrared application with mass spectrometry (MS) that provides a comprehensive Aβ-based profiling approach.

Methods: In the first step, the secondary structure distribution of Aβ peptides is determined using the Immuno-Infrared Sensor setup. The enriched Aβ fraction is eluted and prepared for subsequent MS application. The use of a filter aided sample preparation (FASP) results in a sample that can be used for targeted Aβ analysis as well as for a global MS method for the analysis of Aβ binding partners.

Results: A combined ATR-MS method was developed including the enrichment of Aβ peptides, an elution protocol combined with a compatible MS sample preparation as well as global and Aβ-targeted MS methods. The combination of this methods results in a comprehensive Aβ pattern consisting of Aβ binding partners and different Aβ lengths.

Conclusions: The combination of Immuno-Infrared-Sensor technology and mass spectrometry analysis has the potential to provide added value for Alzheimer's disease diagnostics.
A THIOL CHEMISTRY BASED IMMUNO-INFRARED-SENSOR DETECTING THE AB MISFOLDING IN HUMAN CSF AND BLOOD PLASMA FOR PRECLINICAL DIAGNOSTIC OF ALZHEIMER’S DISEASE

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Aims: Alzheimer’s disease is as neurodegenerative, protein misfolding disease that is caused by the accumulation of plaques containing abnormally folded amyloid beta and tau proteins. The lack of proper clinical diagnostic assays based on biomarkers further delays the start point of intervention. Therefore, it is crucial to develop a diagnostic test for the early detection of the progressive misfolding of amyloid β and tau. We have therefore developed a diagnostic CSF and blood test that monitors the secondary structure distribution of amyloid beta as an biomarker for the onset and progress of AD.

Methods: The immune-infrared-sensor is based on the immobilization of antibodies against amyloid β on chemically functionalized internal reflection elements for ATR-FTIR spectroscopic secondary structure analysis of the bound analyte, the amyloid β protein. The sensor enables the extraction of every amyloid β isoform from complex media such as blood plasma or spinal fluid (CSF) and to determine the secondary structure distribution by ATR-FTIR-spectroscopy. To further improve the sensitivity and specificity of the Alzheimer’s test, we are currently working on thiol-based surface chemistry leaned on the very well established SPR techniques for a stable and highly reproducible binding of antibodies as new progress towards chip development.

Results: Here we present the results obtained by the thiol based functionalization. We already observed performances with thiol functionalized ATR crystals that are at least equal to performances of the sensor obtained in four independent studies (Essen1, Amsterdam2, BioFINDER3, ESTHER3).

Conclusions: Thiol-based surface chemistry leaned on the very well established SPR techniques will be addressed as new progress towards chip development.
THE NEURONAL PENTRAxin RECIPTOR (NPTXR) AS A CANDIDATE BIOMARKER OF SYNAPTIC DISRUPTION IN ALZHEIMER'S DISEASE.

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Aims: Alzheimer's disease (AD) is a progressive and multifactorial disease, leading to the death of the patient. Early deficits cognitive symptoms have molecular background closely related to synaptic loss and dysfunctions. Interestingly, in AD synaptic pathology, pre and postsynaptic proteins were detected in human cerebrospinal fluid (CSF). It has been suggested that one of them was the neuronal pentraxin receptor (NPTXR). NPTXR is involved in synaptic maturity and plasticity influencing synaptic transmission, especially in glutamatergic neurons. This candidate biomarker of synaptic dysfunction may have an essential role in synaptic transmission and modulation of memory processes. In our investigation, the purpose was the quantitate assessment of NPTXR in the CSF and evaluation of the potential usefulness of this protein in the diagnosis of AD patients.

Methods: The study included 15 patients with AD and 15 non-demented controls. The CSF levels of NPTXR and classical AD biomarkers, such as Aβ-42, Aβ-42/Aβ-40, Tau and pTau181 were assessed by commercially available immunoenzyme assays.

Results: We showed a significantly higher CSF concentration of NPTXR in AD patients compared to non-demented controls. Moreover, NPTXR level negatively correlated with tau protein in AD patients.

Conclusions: Our results suggest that Neuronal Pentraxin Receptor may be a promising biomarker of synaptic dysfunction in AD patients. Future research concerning NPTXR is necessary to better understanding the role of the synaptic proteins in early pathological processes of the disease.
A NEW, MULTIPLEX, BLOOD-BASED BIOMARKER ASSAY FOR ALZHEIMER’S DISEASE

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Aims: Alzheimer’s disease (AD) biomarker profiling performed in biofluids use manual or fully automated (random access) immunoassays, including one or more antibodies in the assay design. Differences in clinical performance between various technologies are driven partly by assay precision. With the emerging availability of new technologies to evaluate AD biomarkers, precision qualified multiplex assays developed using well-characterized critical raw materials are expected to become an important part of the assessment process in order to increase acceptance for use in clinical routine or as outcome measures for clinical trials.

Methods: A new, high-throughput, nanosensor platform available at MagArray CLIA laboratory was used for biomarker assay development. The Giant Magneto Resistance (GMR) technology allows for multiplex detection of proteins in different sample types in parallel with ultra-high sensitivity.

Results: Different proteoforms of the most important hallmarks of AD (e.g., tau, phospho-tau, β-amyloid) were quantified in various biological fluids and preliminary results are presented along with those obtained using a proprietary assay incorporating a novel approach. Following optimization of our current assays, we will verify the diagnostic accuracy of each assay to detect brain amyloid in cognitively normal, MCI and AD subjects. The blood samples for validation studies are obtained from academic partners and include corresponding CSF biomarker profiles and/or amyloid PET statuses.

Conclusions: MagArray platform and GMR technology, together with the new assays/methods presented here are uniquely positioned to help the healthcare community deliver better outcomes through improved detection of AD biomarkers early in the disease process. This study was funded by ADDF’s Diagnostics Accelerator Program.
NEUROPHYSIOLOGICAL BIOMARKERS PARALLEL GLUCOSE HYPOMETABOLISM AND HYPOPERFUSION IN MCI AND ALZHEIMER’S DISEASE PATIENTS

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Aims: The aim of the study is to demonstrate that neurophysiological biomarkers provide robust, reliable, cost-effective tools for tracking disease progression and assessing efficacy of interventions for Alzheimer’s disease (AD) by comparison of EEG biomarkers to two well established neuroimaging modalities.

Methods: Resting state EEG with 5 minutes eyes open and 5 minutes eyes closed was acquired from a cohort of Alzheimer’s disease and mild cognitive impairment subjects compared to age and gender matched healthy controls and benchmarked against 18F-fluorodeoxyglucose positron emission tomography, a measure of cerebral metabolic rates of glucose and arterial spin labeling, an assessment of cerebral blood perfusion.

Results: The hallmark “slowing” of EEG was observed as an enhancement of slow bandwidths and a suppression of higher bands in the AD cohort compared to healthy controls. Significant correlations were identified between EEG metrics and regions of hypometabolism and hypoperfusion in AD patients, including the cingulate gyrus and precuneus, two important structures to identify and analyze when reviewing brain images obtained in patients with cognitive impairment.

Conclusions: The data suggest that metrics from simple resting state EEG parallel hypometabolism and hypoperfusion associated with cognitive decline and Alzheimer’s disease progression. The results support the use of a resting state EEG pharmacodynamic endpoint as a proxy for neuronal activity to evaluate efficacy of interventions in clinical studies focused on Alzheimer’s disease.
IS EPISODIC MEMORY PERFORMANCE RELATED TO POST-TASK BRAIN ELECTRICAL ACTIVITY IN COGNITIVELY UNIMPAIRED SENIORS AND PATIENTS WITH MILD COGNITIVE IMPAIRMENT?

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Aims: Experiments on electroencephalographic (EEG) oscillations in aged people typically include blocks of cognitive tasks with a few minutes of interval between them. The present exploratory study tested whether task performance might be related to spontaneous dominant EEG alpha (8-12 Hz) rhythm in posterior regions a few minutes after task completion in cognitively unimpaired (CU) seniors and patients with amnestic mild cognitive impairment (aMCI).

Methods: Resting state with eyes closed EEG activity (rsEEG) in 30 CU and 40 matched aMCI seniors was recorded 5 minutes after performing a delayed recall episodic memory (EM) task. Cortical sources of rsEEG were estimated by e-LORETA with a focus on alpha rhythms as an index of general cortical arousal. The relationship between task performance and posterior rsEEG alpha source activity was assessed for each group, as well as the differences of the correlation coefficients between groups.

Results: No significant correlations between task performance and posterior alpha activity were found for the aMCI group. However, reaction time and posterior alpha activity positively correlated in the CU group, with better performance related to lower alpha activity. Furthermore, for these significant correlations, there were differences between groups in the correlation coefficients.

Conclusions: These results suggest that only in CU seniors, an intense engagement in memory tasks may influence brain arousal for some minutes after the end of cognitive tasks. Future cross-validation studies in CU and aMCI groups should repeat the present experiments including (1) a resting-state EEG recording before EM tasks and (2) post-task resting-state EEG recordings at different lags.
DIFFERENTIAL DIAGNOSIS BETWEEN NON-DEGENERATIVE MILD COGNITIVE IMPAIRMENT AND EARLY ALZHEIMER'S DISEASE THROUGH A TABLET-BASED DIGITAL BIOMARKER

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Aims: To assess the performance of ALTOIDA-iADL for the differential diagnosis between patients with non-degenerative mild cognitive impairment (MCI) and prodromal (pAD) and mild (mAD) Alzheimer’s disease.

Methods: ALTOIDA-iADL is a 10-minute administrable cognitive test, which assesses activities of daily living in the form of an augmented reality game. The task consists of placing and finding virtual objects in a real environment and provides a final score (the NeuroMotor Index; NMI). The NMI is obtained by weighting multi-modal information such as hands’ micromovements, screen touch frequency, reaction times or navigation trajectory, among others. We included 65 participants and classified according to cerebrospinal fluid (CSF) AD biomarkers: MCI (n=23; age: 68.1; MMSE: 26.4), pAD (n=28; age: 70.1; MMSE: 24.6) and mAD (n=14; age: 70.6; MMSE: 20.7).

Results: The NMI allowed differentiating between the amyloid-negative (Aβ-) and amyloid-positive (Aβ+) groups (p<0.01). Furthermore, we found differences between the MCI group and the pAD (p<0.01) and mAD (p<0.01) groups (Fig. 1). ROC curves showed good diagnostic accuracy of the NMI in the discrimination between the Aβ- and Aβ+ (AUC=0.821; p<0.01), MCI and pAD (AUC=0.846; p<0.01) and MCI and mAD (AUC=0.832; p<0.01). The NMI did not discriminate between the pAD and mAD (AUC=0.574; p=0.57) groups (Fig. 2). The NMI correlated with CSF NfL levels (r=-.529; p<0.05) and the MMSE (r=.372; p<0.01).
Conclusions: ALTOIDA-iADL is useful in the differential diagnosis between patients with non-degenerative MCI and prodromal and mild Alzheimer’s disease. Its performance is related to the degree of impairment in cognitive screening tests and with biomarkers of axonal damage/neurodegeneration.
DUAL-TASK PERFORMANCE IS ASSOCIATED WITH AMYLOIDOSIS IN COGNITIVELY HEALTHY ADULTS


Aims: Preclinical Alzheimer’s disease (AD) provides an ideal target for the study and implementation of interventions and strategies aimed at delaying, mitigating, and preventing AD; however, it is difficult to identify efficiently and cost-effectively. Recent findings have suggested that cognitive-motor dual-task paradigms may provide additional inference. This study aimed to investigate the relationship between dual-task performance and amyloidosis, suggestive of preclinical Alzheimer’s disease, and whether dual-task performance provides additional information beyond a cognitive composite, to help in the identification of amyloidosis.

Methods: Data from 52 cognitively healthy adults were obtained for this cross-sectional study. The data included demographics, amyloid standardized uptake value ratio (SUVR) obtained via florbetapir-PET, neuropsychological testing, apolipoprotein E (APOE) genotype, and dual-task performance measures. Data were analyzed via hierarchical multiple linear regression or logistic regression, controlling for age, education, and APOE genotype.

Results: There was a moderate relationship (rs>.30) between motor and cognitive dual-task effects (DTE) and amyloid SUVR (ps<.042). A strong relationship (r=.58) was found between combined DTE (cDTE), a measure of automaticity derived from dual-task performance, and amyloid SUVR (p<.001). Additionally, cDTE showed promise in its unique contributions to amyloid SUVR, accounting for 7.8% of amyloid SUVR variance above cognitive composite scores (p=.018).

Conclusions: Dual-task performance using the combined dual-task effect, a measure of automaticity, was a moderate predictor of cerebral amyloidosis, which suggests that it has utility in the screening and diagnosis of individuals for preclinical AD. Further research is warranted.
POSTERS

DIGITAL CLOCK DRAWING PERFORMANCE REVEALS SUBTLE MOTOR IMPAIRMENTS THAT ASSISTS DIFFERENTIATION OF EARLY-STAGE DEMENTIA DUE TO ALZHEIMER'S DISEASE, DIFFUSE LEWY BODIES AND PARKINSON'S DISEASE.

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Aims: To compare digital clock drawing performance between Alzheimer's disease, Diffuse Lewy Bodies and Parkinson's Disease.

Methods: Neurodegenerative disorders such as Alzheimer's disease (AD), Diffuse Lewy Bodies (DLB), and Parkinson's Disease (PD) are among the most prevalent conditions in aging and are a source of significant disability. The Linus DCTclock™ assessment enables early detection of cognitive deficits in individuals with confirmed AD neuropathology even when standard neuropsychological assessment fails to identify deficits by employing machine learning algorithms, thus reducing bias from subjective judgement in test scoring (Souillard-Mandar et al, 2015; Rentz et al, 2021). Importantly, in addition to scoring the final produced drawings, the Linus DCTclock™ captures the drawing process, with key metrics that quantify distinct cognitive metrics (e.g., memory, executive and visuospatial abilities), and motor metrics (e.g., response time, drawing efficiency, tremor).

Results: While cognitive impairments may be present in each of these conditions, consideration of specific motor impairments can provide earliest signs of PD and DLB. Therefore, the DCTclock™ enables differentiation of PD or DLB from dementia due to AD.

IDENTIFICATION OF SPEECH CHARACTERISTICS AND DETECTION OF ALZHEIMER'S DISEASE USING ACOUSTIC FEATURES FROM STORY RECALL TASK

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Aims: Speech impairment is one of the early symptoms of Alzheimer's Disease (AD) and has a great potentiality as an early marker of AD diagnosis. Our work attempts to identify speech characteristics of AD patients and exploit them to detect AD.

Methods: We recorded brief interview and story recall task generated by 94 healthy participants (mean age 73.4) and 65 AD patients (mean age 78.9). Participants were interviewed about their daily life and performed repetition and recall of two modified well-known fairy-tale to impose cognitive load. The extraction process was done using the Python library My-Voice-Analysis 4 and openSMILE toolkit. The investigation of the characteristics of AD patients and feature selection was carried out with ANOVA. The classification was carried out using Random Forest classifier and 10-fold cross-validation was implemented.

Results: The analysis of speech samples reveals that AD patients produce a smaller number of syllables, shorter duration of speech and lower speech rate which indicates shortness and slowness of speech. Monotonous loudness is observed with the reduced number of loudness peak per second and the gradual slope of loudness. Low Harmonics-Noise-ratio of AD patients indicates a noisy or hoarse characteristic of voice. We obtained a classification accuracy of 91.1% in discriminating individuals with AD from controls. Results using 10-fold cross-validation achieved an accuracy of 89.8%.

Conclusions: Our work describes specific speech characteristics of AD patients using story recall task which elicits speech impairments with cognitive demands and highlights the applicability of spontaneous speech as a detection method of AD using only acoustic features.
POSTERS

PRIMARY PROGRESSIVE APRAXIA AS A FORM OF PRESENTATION OF ALZHEIMER’S DISEASE: DESCRIPTION OF THREE CASES.

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Aims: Primary Progressive apraxia (PPA) is an unusual presentation of some neurodegenerative syndromes. It is defined as a slowly progressive apraxic syndrome in absence of dementia. In some cases, it has been associated with some degree of language impairment (progressive aphasia or apraxia of speech). PPA has been related to Pick’s disease pathology, four-repeat tau pathology, specially corticobasal syndrome, Alzheimer’s Disease and Frontotemporal dementia. In addition, progressive apraxia of speech consists on a primary motor speech disorder and it has also been related to four-repeat tau pathology.

Methods: We describe three patients with a progressive cognitive disorder, with apraxia as main symptom

Results: Symptoms began 5-6 years ago, two of them with slowly progressive speech difficulty and words evoking, with preserved understanding and repetition. Third one first developed gait disturbance and falls. All three developed problems with writing, dressing, cooking or using cutlery. All of them had some oculomotor disturbance, with limited vertical gaze and saccades, with no extrapyramidal or motoneuron signs. Neuropsychological assessment showed severe motor, ideomotor, visuoconstructive and speech apraxia, with relatively preservation of episodic memory. MRI showed diffuse symmetric atrophy. Two of them underwent lumbar puncture, with low Aβ 1-42 levels and high total and phosphorylated tau levels, suggesting Alzheimer’s pathology.

Conclusions: We present 3 cases of primary progressive apraxia (including speech apraxia) without significant memory impairment. Imaging was compatible with a corticobasal syndrome and biomarkers with an Alzheimer’s Disease. To our knowledge, few cases of primary progressive apraxia have been reported in literature.
Aims: To characterise the differences and similarities between patients with clinically diagnosed Alzheimer’s dementia (AD) or mixed dementia (MD) of the Alzheimer’s plus vascular type, from a naturalistic cohort. We aimed to study multiple biomarkers, vascular factors, and key demographic and clinical features.

Methods: 135 patients with AD and 108 with MD were selected from the naturalistic multicentre cohort MemClin. A subsample of 83 AD and 51 MD patients had available cerebrospinal fluid (CSF) biomarkers. All patients had visual rating of medial temporal lobe atrophy (MTA) and white matter hyperintensities (WMHs) and neuropsychological testing. T-test and Chi² was used for group differences, as well as ANCOVA to correct for covariates when applicable. Visual ratings were age adjusted.

Results: Both groups were comparable in terms of MMSE, frequency of pathological CSF amyloid-beta-42 or phosphorylated tau and immediate word recall. The MD group had a higher frequency of pathological MTA, had shorter education, and was older, but performed better at delayed recall as compared with the AD group. As expected, the MD group also had more WMHs and cerebrovascular events.

Conclusions: In our cohort, MD patients showed more MTA than AD patients, at same degree of AD pathology and global cognitive impairment. Better performance in delayed recall may help discriminating MD from AD, but further research is needed to improve the differential diagnosis between these two very common disorders in naturalistic settings.
LONGITUDINAL ASSESSMENT OF NOVEL WHITE MATTER IMAGING MEASURES OF NEUROINFLAMMATION, AXONAL DENSITY AND DEMYELINATION IN LARGE SCALE MULTICENTER ADNI

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Aims: Three recent quantitative white matter (WM) measures from diffusion MRI (dMRI) were analyzed: i- free-water, a marker of neuroinflammation ii- apparent fiber density, a marker of axonal integrity iii- tissue radial diffusivity, a marker of myelin content. Our objective was to establish the dynamics of these three markers over 2 years in ADNI.

Methods: All ADNI cohorts with dMRI at 3-, 6-, 12- and 24-months were included, which resulted in 51 NC, 78 MCI and 37 AD. In the MCI group, 15 subjects converted to AD over the 24 months. From dMRI, free-water, apparent fiber density and tissue radial diffusivity maps were computed in WM bundles altered in AD. Age, sex, apolipoprotein E4 status, intracranial volume and total WM hyperintensities volume were used as covariates.

Results: In AD patients, free-water was increased by 7.7, 11.1 and 16.7% at 6, 12 and 24 months. In AD patients, fiber density in the fornix was decreased by 2.3, 4.3 and 4.4% at 6, 12 and 24 months. Fornix reductions in fiber density at 24 months were larger in ADvsNC and MCIvsNC. At 24 months, tissue radial diffusivity increase in the fornix was higher in ADvsNC. MCI converters to AD had 11% higher free-water, 14% lower fiber density and 10% higher radial diffusivity compared to MCI-stable.

Conclusions: In MCI, the three biomarkers in the fornix, especially fiber density, may be predictive of conversion to AD. In clinical trials, stabilizing (or reversing decline) fiber density and tissue radial diffusivity measures would suggest tissue repair, strengthened axons and potential remyelination.
GERMAN VALIDATION OF THE NTG-EDSD AND DSQIID FOR SCREENING AND DIAGNOSIS OF ALZHEIMER DISEASE IN PEOPLE WITH DOWN’S SYNDROME

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Aims: Due to a triplication of the amyloid precursor protein (APP) gene on chromosome 21, people with Down’s Syndrome (DS) are at high risk of developing an Alzheimer type dementia (DS-AD). Internationally established tools for screening and diagnosing DS-AD are not available in German. Therefore, we conducted a German validation study of the DSQIID (Dementia Screening Questionnaire for Individuals with Intellectual Disabilities) and NTG-EDSD (NTG-Early Detection Screen for Dementia).

Methods: 71 participants were assessed clinically and neurospychologically and categorized according to ICD-10 and DSM-V criteria for dementia. DSQIID/NTG-EDSD sensitivity were first determined for an vs. no cognitive decline, whereas specificity analyses differentiated MCI/AD and secondary/unknown etiologies.

Results: The prevalence of cognitive decline in our sample was 52.1% (n=37; AD: 21.1%, MCI: 9.9%, secondary: 12.7%, unknown: 8.5%) Patients with cognitive decline showed markedly higher scores in both DSQIID (median(range): 14.0 (0-46) vs. 2.1 (0-21), p<0.001) and NTG-EDSD (18.0 (1-49) vs. 3.1 (0-23), p<0.0001). However, the established DSQIID cutoff-value (20 points) yielded low sensitivity (53.3%) and specificity (47.1%) for DS-AD. With the screening tool NTG-EDSD, Cognitive decline was detected with good sensitivity (94.6%) and acceptable specificity for DS-AD (71.4%) at a cutoff-value of 7.

Conclusions: In summary, the German NTG-EDSD is a valuable tool to detect cognitive decline in people with DS. However, the specificity for DS-AD of both the NTG-EDSD and the DSQIID are moderate to poor, emphasizing the need for detailed investigations in at-risk patients. To maximize sensitivity for MCI, caregiver-reported disturbances of speech/memory as well as self-reported deficits should prompt a detailed investigation.
A NEW TOOL TO ACCESS THE EFFECT OF INTRACEREBRALLY ADMINISTERED ANTI-AMYLOID-B COMPOUNDS

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Aims: Until now, there is no standardized method to examine Aβ after targeted delivery and distribution of test substances in the brain using mini-osmotic pumps. The aim of the present study was to develop a new tool to quantify the local distribution of Aβ plaques after intracerebral infusion of compounds.

Methods: We developed a toolbox to quantify Aβ plaques in relation to intracerebral injection channels using Zeiss AxioVision® and Microsoft Excel® software. 50-day-old C57BL/6J received PBS intracerebrally via ALZET® mini-osmotic pumps (model 2006) that were implanted for 42 days by stereotactic surgery. At the age of 100 days, brains were collected for immunhistological analysis.

Results: Our approach delivers a more accurate quantification of Aβ plaques (number, size and coverage) after long-term intracerebral injection. Alternative imaging software’s do not provide specific approaches for situations with lost tissue and injection trajectories. The tool provides classification of Aβ plaques in pre-defined distance groups using two different approaches.

Conclusions: To conclude, this new analytic tool is advantageous for the analysis of experimental long-term continuous intracerebral compound infusions using ALZET® pumps. This method helps to generate reliable data for Aβ characterization and distribution of experimental compounds.
A VISIONOMOTOR ABILITY-BASED POTENTIAL SCREENING METHOD FOR ALZHEIMER'S DISEASE?

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Aims: Alzheimer's disease (AD) is the primary cause of dementia in older adults. Latest results presume that impairment in visuomotor function also appears in the initial stages of the disease. To support a more accessible and extensive diagnostic opportunity for AD, we developed a visuomotor ability-based computerized screening method.

Methods: 52 AD patients underwent neuropsychological assessment including Addenbrooke Cognitive Examination (ACE). Cognitive scores were extensively analyzed. Based on the results, our group developed a computerised test battery (Precognize). It is based on the Trail-making test and used for detecting and analyzing mouse movements. To evaluate the accuracy of our tool, we examined control participants (N=24) and patients (N=9) suffering from mild cognitive impairment or AD. They also underwent structural and functional MRI acquisition and neuropsychological evaluation.

Results: Visuospatial score showed strong negative correlation with disease duration (r=-0.73; p<0.001). Significant difference appeared in mouse movements between the control and patient group (p=0.019). Prominent correlation appeared between ACE visuospatial subscores and mousemove parameters (r= -0.39 p=0.037). Significant negative correlation presented between Precognize results and the cortical volume of both hemispheres (r= -0.59; p=0.006). Functional MRI results showed alteration of connectivity of the fronto-parietal network between the control and patient group.

Conclusions: Results from our research show that testing of visuospatial abilities with electronic algorithms might hold important screening potential for large populations in the early identification of cognitive decline. Our test battery seems promising since the extracted data correlate well with the results of neuropsychology and neuroimaging.
Aims: Sharing brain imaging data within the open science framework comes with many chances, but also challenges and limitations.

Methods: To address these issues, we have formed a Cluster of “big-imaging-data” projects from JPND, Horizon2020, and IMI. These projects are supplemented by National Neuroimaging Platforms of France, Italy and Germany and one of the largest national multicentric imaging biomarker consortium, the Swedish BioFINDER Study. This Cluster will weave together large European projects of different entities (Consortia, Networks, and Platforms) for a common greater goal: deploying latest advances in data science, computing, and imaging technologies to develop imaging biomarkers supporting personalized diagnostics and treatments in brain disorders.

Results: The workplan includes a survey among the peer group of principle investigators in European brain imaging projects and a consensus meeting to formulate positions and viewpoints on the topics. We will present the results of the survey at the ADPD meeting and hope to spur a broader discussion on one of the most important topics in science today: sharing data under the umbrella of open science.

Conclusions: More details can be seen here: https://www.ebra.eu/ecib-cluster/
POTENTIAL CONTRIBUTION OF MUTATIONS IN UROKINASE-TYPE PLASMINOGEN ACTIVATOR (PLAU) AND B-SITE APP-CLEAVING ENZYME 1 (BACE1) TO SEMANTIC DEMENTIA PHENOTYPE

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Aims: Our study aimed at identifying the genetic variants potentially contributing to the early-onset semantic dementia phenotype developed at the age of 64. As there was family history of dementia and episodic memory deficit accompanied profound semantic loss, atypical Alzheimer’s disease (AD) was also considered.

Methods: Whole-genome sequencing has been performed, followed by functional analyses of selected variants on the mRNA and protein level in the patient’s primary skin fibroblasts.

Results: According to ACMG criteria only very rare variants of unknown significance (VUS) have been identified: a nonsense variant c.366C>A/p.Cys122* in Plasminogen Activator, Urokinase (PLAU), a missense variant c.944C>T/ p.Thr315Met in β-site APP-cleaving enzyme 1 (BACE1), and c.3436C>T/ p.Arg1146Cys variant in mtDNA polymerase gamma (POLG) - along with the established moderate penetrance variants: APOE3/APOE4 and MAPT H1/H1 haplotype. Patient-derived fibroblasts showed reduced PLAU mRNA and protein levels due to nonsense-mediated mRNA decay (NMD), while BACE1 mRNA and protein levels were increased as compared to control fibroblasts.

Conclusions: This is the first report of PLAU variant with the confirmed underlying mechanism of haploinsufficiency in any known disease phenotype. p.Cys122stop variant could possibly cause PLAU dysfunction. Our results suggest that rare mutations in PLAU and BACE1 genes should be considered in cases of early-onset dementia. Our data support an oligogenic pathogenicity model for early-onset dementias.
AB PROFILES GENERATED BY ALZHEIMER’S DISEASE CAUSING PSEN1 VARIANTS DETERMINE MUTATION PATHOGENICITY AND PREDICT AGE AT DISEASE ONSET

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Aims: Familial Alzheimer’s disease (FAD), caused by mutations in Presenilin (PSEN1/2) and Amyloid Precursor Protein (APP) genes, is associated with early ages at AD onset (AAO). AAOs differ markedly between subjects carrying different mutations. Why certain mutations manifest several decades earlier than others is not well understood. Pathogenic mutations affect the protease (PSEN/γ-secretase) and substrate (APP) that generate amyloid β (Aβ) peptides. Altered Aβ metabolism has been long associated with AD pathogenesis, with absolute or relative Aβ42 increments most commonly connected to the disease severity. However, analyses studying Aβ42 increments and AAO are inconsistent and there is no consensus on what Aβ species best reflect the mutation pathogenicity. We investigated this central aspect of AD pathophysiology by applying both biased and unbiased approaches. We tested a mechanism-driven hypothesis that links mutation-induced destabilization of γ-secretase-Aβn interactions with alterations in Aβ profile composition and AAO to uncover the relationships between Aβ profile composition and AAO.

Methods: We analysed Aβ profiles generated from 25 PSEN1 mutations associated with a broad range of AAO (i.e. from 24 to 65 years)

Results: Our studies demonstrate linear correlations between mutation-driven alterations in Aβ profiles and AAO, offer predictive value in the assessment of ‘unclear’ PSEN1 variants and importantly, provide quantitative support for therapeutic strategies aimed at shifting Aβ profiles towards shorter ones.

Conclusions: Collectively, these analyses strongly support the critical implication of the alterations in the relative amounts of Aβ peptides in the FAD pathogenesis, their role in the determination of the AAO and provide evidence in favour of the amyloid cascade hypothesis.
DYSREGULATED EXPRESSION LEVELS OF APH1B IN PERIPHERAL BLOOD ARE ASSOCIATED WITH BRAIN ATROPHY AND AMYLOID-B DEPOSITION IN ALZHEIMER’S DISEASE

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Aims: The interaction between brain and periphery might play a crucial role in the development of Alzheimer’s disease (AD).

Methods: Using blood transcriptomic profile data from two independent AD cohorts, we performed expression quantitative trait locus (cis-eQTL) analysis of 29 significant genetic loci from a recent large-scale genome-wide association study to investigate the effects of the AD genetic variants on gene expression levels and identify their potential target genes. We then performed differential gene expression analysis of identified AD target genes and linear regression analysis to evaluate association of differentially expressed genes with neuroimaging biomarkers.

Results: cis-eQTL analysis identified and replicated significant associations in seven genes (APH1B, BIN1, FCER1G, GATS, MS4A6A, RABEP1, TRIM4). APH1B expression levels in blood increased in AD and were associated with entorhinal cortical thickness and global cortical amyloid-β deposition.

Conclusions: An integrative analysis of genetics, blood-based transcriptomic profiles, and imaging biomarkers suggests that APH1B expression levels in blood might play a role in the pathogenesis of AD.
Aims: Established genetic risk factors for Alzheimer’s disease (AD) account for only a portion of AD heritability. The aim of this study was to identify novel associations between genetic variants and AD-specific brain atrophy.

Methods: We conducted genome-wide association studies for brain magnetic resonance imaging measures of hippocampal volume and entorhinal cortical thickness in 2,643 Koreans meeting the clinical criteria for AD (n=209), mild cognitive impairment (n=1,449) or normal cognition (n=985).

Results: A missense variant, rs77359862 (R274W), in the SHANK-associated RH Domain Interactor (SHARPIN) gene was associated with entorhinal cortical thickness (p=5.0×10^{-9}) and hippocampal volume (p=5.1×10^{-12}).
It revealed an increased risk of developing AD in the mediation analyses. This variant was also associated with amyloid-β accumulation (p=0.03) and measures of memory (p=1.0×10⁻⁴) and executive function (p=0.04). We also found significant association of other SHARPIN variants with hippocampal volume in the Alzheimer's Disease Neuroimaging Initiative (rs3417062, p=4.1×10⁻⁶) and AddNeuroMed (rs138412600, p=5.9×10⁻⁵) cohorts.
Further, molecular dynamics simulations and co-immunoprecipitation indicated that the variant significantly reduced the binding of linear ubiquitination assembly complex proteins, SHPARIN and HOIL-1 Interacting Protein (HOIP), altering the downstream NF-κB signaling pathway.
Conclusions: These findings suggest that SHARPIN plays an important role in the pathogenesis of AD.
CAUSAL EPIGENETIC ASSOCIATION BETWEEN AD AND CPG METHYLATION CHANGES

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Aims: DNA methylation (DNAm) is one of the epigenetic mechanisms, which alters gene expression without changing DNA sequence. Recent studies revealed that a few DNAm markers have a value in early-stage diagnosis for AD and DNAm from blood is emerged as an easily achieved biomarkers. In this study, we tried to identify the causal relationship between AD and DNAm markers to improve the understanding the role of DNAm as a biomarker in detection AD in early stage.

Methods: We examined the causal effect of DNAm on AD and mild cognitive impairment (MCI) using two sample Mendelian randomization (2SMR). Publicly available summary statistics of AD/MCI and methylation quantitative loci (mQTL) information in European population were utilized in 2SMR analysis.

Results: 2SMR revealed 8 significant CpG sites (on HLA-DRB6, HLA-DRB1, STAG3, MS3A3, FAM63B, APOC1, and BCL3) has causal effect on AD. Among them, causality of two CpG sites were still significant after adjusting pleiotropic effect (β_IVW=-6.49 (P<0.001), β_Egger=-3.19 (P=0.003) for cg0021215 and β_IVW=-5.99 (P<0.001), β_Egger=-3.19 (P=0.023) for cg00553149). MCI risk decreases as cg00212031 (on Y chromosome) hypermethylated (β_IVW=-3.56 (P<0.001) and β_Egger=-3.19 (P=0.046)) which is interest considering MCI prevalence is higher in women. Three CpG sites (cg00103771, cg00553149 and cg02771260) from AD 2SMR were significantly associated with MCI, but 2SMR estimates were not available due to not enough number of SNPs.

Conclusions: In this study, causality of AD/MCI to DNAm changes were investigated. AD had an effect on 8 CpGs located in AD associated genes and MCI was causally associated with CpG on Y chromosome.
POSTERS

RATE OF ALZHEIMER'S DISEASE PROGRESSION ANALYSIS IN PATIENTS CARRYING RISK CONFERRING ALLELES

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Aims: Alzheimer's disease (AD) patients show varying rates of cognitive decline after onset. We report here results from a genetic association of rate of AD progression using previously published single nucleotide polymorphisms (SNPs) that confer risk for AD onset.

Methods: We used Integrated Alzheimer's Disease Rating Scale (iADRS) as a clinical endpoint and utilized regional Tau and cortical thickness measures for biomarker validation. In total, we tested 34 AD risk SNPs reported in a recent Genome-Wide Association Study (Bellenguez et al., 2020). The effect of each AD risk allele on iADRS progression was evaluated using a mixed effect repeated measures model (MMRM) adjusting for age, sex, education, prior AD medications and initial AD severity and results were combined in a meta-analysis using METAL. We also explored association of risk alleles with imaging endpoints by analysis of covariance model controlling for the effect of age, sex, and APOE4 status.

Results: Only SNP rs6733839 in the gene BIN1 showed association with rate of cognitive decline ($p=1.6 \times 10^{-3}$) among the 34 tested SNPs. Furthermore, rs6733839 also associated with significantly higher baseline global Tau accumulation in temporal part of the brain.

Conclusions: Taken together, our findings suggest that patients with risk SNP in BIN1 rs6733839 progress faster and show higher Tau accumulation and decreased cortical thickness.

Clinical trial cohort used in this study

<table>
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<tr>
<th>Clinical Trial (ClinicalTrials.gov ID)</th>
<th>n (Placebo Arm MMRM)</th>
<th>n (Flortaucipir-PET)</th>
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<tr>
<td>EXPEDITION-3(NCT01900665)</td>
<td>816</td>
<td>182</td>
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<tr>
<td>NAVIGATE-AD(NCT02791191)</td>
<td>--</td>
<td>223</td>
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<tr>
<td>AMARANTH(NCT02245737)</td>
<td>267</td>
<td>101</td>
</tr>
<tr>
<td>DAYBREAK(NCT02783573)</td>
<td>315</td>
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</table>

We used Integrated Alzheimer's Disease Rating Scale (iADRS) as a clinical
IDENTIFYING GENETIC LOCI FOR COGNITIVE PRESERVATION IN THE MIDWESTERN AMISH

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Aims: Genetic studies of Alzheimer disease (AD) have focused primarily on identifying loci and genes that increase the risk of disease rather than those that decrease the risk. By finding loci that decrease risk of dementia, we may identify biological pathways and processes that help delay or even prevent dementia. Here we perform both a genome-wide association study (GWAS) as well as multiple genetic linkage analyses to identify loci associated with cognitive preservation. The phenotype of interest is cognitive resistance or resiliency, which is the lack of AD pathological markers or the lack of AD cognitive symptoms in the presence of AD pathologies, respectively.

Methods: To help increase our ability to detect possible rare variants, we have studied cognitive resistance/resilience the Midwestern Amish, an isolated founder population of European descent, for this analysis. The R package GENESIS was used for the GWAS to help incorporate the extensive genealogical data we have on the Midwestern Amish. MERLIN was used for linkage analysis across sub-pedigrees for both two-point and multipoint nonparametric linkage analyses.

Results: We were able to reconstruct a 15-generation 8,222 individual single pedigree to help inform both our association and linkage analyses. Over 250,000 SNPs were used across the genome for our GWAS and two-point linkage analyses, which were pruned to subsets of 167,228 and 5,294 SNPs for the multipoint linkage analyses using the SNPrelate package.

Conclusions: Suggestive loci are seen on chromosomes 3, 5, 7, 11, 13, 16 in both GWAS and linkage preliminary results. Fine mapping and parametric linkage analyses are ongoing.
Aims: Develop novel approach for Alzheimer’s (AD) and Parkinson’s Disease (PD) genome-wide association studies (GWAS) in UK Biobank (UKB).

Methods: UKB is valuable for AD/PD gene discovery by using parental information as a proxy phenotype. We integrated offspring and parental data to create an improved phenotype (Figure-1). GWAS considered European ancestry participants, linear mixed modelling on case-control status (LMM-BOLT), and variants with imputation scores >=0.3 and effect allele frequencies >=0.05% and >=1% for AD and PD respectively. For AD, a scalar phenotype (Jansen et al. 2019) was also tested and integrated with case-control findings (selecting minimum P-value per variant).

Results: Demographics are in Table-1. We found 17 AD and 5 PD novel risk loci (Figure-2-3). Many AD/PD loci available in the largest independent AD/PD GWAS (Kunkle/Nalls et al. 2019) were replicated at nominal significance (Figure-4).

<table>
<thead>
<tr>
<th>UKB GWAS</th>
<th>Participants (N)</th>
<th>Cases (N)</th>
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</thead>
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<tr>
<td>AD – Schwartzentruber-2021</td>
<td>408,942</td>
<td>53,042 (13.0%)</td>
</tr>
<tr>
<td>AD – Novel-approach</td>
<td>438,080</td>
<td>61,350 (14.0%)</td>
</tr>
<tr>
<td>PD – Nalls-2019</td>
<td>451,391</td>
<td>18,510 (4.10%)</td>
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<tr>
<td>PD – Novel-approach</td>
<td>451,006</td>
<td>21,706 (4.81%)</td>
</tr>
</tbody>
</table>

Table-1. Demographics for novel and largest prior AD/PD GWAS in UKB.
Figure 1. Schematic overview of novel approach. PD approach is equivalent except filtering for age.
Figure 2. Manhattan plots. Green and red dots respectively indicate suggestive and genome-wide significance. Top variants are annotated with nearest gene. Blue text indicates novel hits with regard to prior largest UKB GWAS.
Figure 3. Scatter plots of novel versus prior significances.

Figure 4. Novel AD hits in independent AD/PD GWAS. Kunkle-2019 contained 35,274 cases and 59,163...
controls. Nalls-2019-no-UKB included 28,764 cases and 107,919 controls (these data also excluded 23andMe samples).

**Conclusions:** Our results bring new insights into the genetics of AD/PD and will contribute importantly to gene prioritization by integration with other GWAS samples.
ASSOCIATION OF A CAMK2A GENETIC VARIANT WITH MEMORY IMPAIRMENT AND ALZHEIMER’S PATHOLOGY

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Aims: Dysregulation of Calcium/Calmodulin-dependent kinase alpha (CaMKIIα) is linked to memory impairment and Alzheimer’s disease (AD) pathophysiology. We investigated six single nucleotide polymorphisms (SNPs) in the CaMKIIα gene for association with memory performance and/or postmortem amyloid-β plaques and neurofibrillary tangle (NFT) burden.

Methods: Relationships between CaMKIIα SNPs and learning and delayed recall performance (Rey Auditory Verbal Learning Test) were examined in 801 participants from the AD Neuroimaging Initiative (ADNI; age range: 55-90; 45% women; 93% Caucasian, 36% MCI, 6% AD dementia). Relationships between CaMKIIα SNPs and odds of higher amyloid-β plaques (CERAD score>1) or NFT (BRAAK score>2) burden were examined among 2,043 post-mortem cases from the National Alzheimer’s Coordinating Center (NACC; age at death: 47-111; 48% women; 97.5% Caucasian, 10% MCI, 71% AD dementia). Covariates included age, sex and APOE4 genotype.

Results: Across cohorts, the rs6869634 SNP related to AD outcomes. In ADNI, rs6869634 GA genotype related to better learning compared to the GG genotype (p=.02) and with better recall compared to GG (p=.001) and AA (p=.03) genotypes. In NACC, the AA genotype related to lower odds of high amyloid burden compared to the GA (p=.02) and GG (p=.051) genotypes and lower odds of high tau burden compared to the GG genotype (p=.048). Significant results do not withstand multiple testing correction.

Conclusions: The CaMKIIα rs6869634 consistently related to learning/memory performance in ADNI and AD pathology in NACC, although the direction of these relationships may differ by disease stage possibly due to overexpression of CaMKIIα as a compensatory reaction to neural injury.
AN IL1RL1 GENETIC VARIANT LOWERS SOLUBLE ST2 LEVELS AND ATTENUATES THE IMPACT OF APOE-Ε4 IN ALZHEIMER’S DISEASE

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Aims: Genetic factors are increasingly recognized as important contributors to the pathogenesis of Alzheimer’s disease (AD). While the expressions of most AD risk genes are enriched in microglia, which lead to microglial dysfunctions and amyloid-beta (Aβ) accumulation, emerging studies suggest that changes of the brain micro-environment—typically the levels of soluble factors—also modulate disease-related pathologic changes. Therefore, understanding the genetic regulation and pathogenic roles of those soluble factors in AD will help clarify the pathophysiological mechanisms of the disease.

Methods: We examined the level changes of soluble ST2 (sST2), a soluble decoy receptor of IL-33/ST2 signaling, in blood and brains of patients with AD, and performed the genome-wide association study (GWAS) for sST2 to understand its genetic regulations.

Results: We showed that elevated blood and brain levels of sST2 are correlated with severe neurodegeneration and Aβ pathologic lesions in patients with AD. Intracerebroventricular injection of sST2 resulted in exacerbated Aβ accumulation and reduced Aβ–microglia colocalization in an amyloidosis transgenic mouse model. Our GWAS results identified a genetic variant in IL1RL1 (the gene that encodes sST2) that is associated with decreased sST2 levels in blood and brains. Furthermore, we demonstrated that the genetic variant reduces the risk of AD and Aβ accumulation through enhancing the activation of microglia and their colocalization with Aβ in female APOE-ε4 carriers.

Conclusions: These findings demonstrate how a genetic variation modulates sST2 levels in the brain milieu to regulate the impact of APOE-ε4 in AD, which facilitates the development of novel intervention strategies for the disease.
Aims: Recent advances in genetic sequencing have enabled comprehensive genetic analyses of human diseases, resulting in the identification of numerous genetic risk factors for heritable disorders including Alzheimer’s disease (AD). Such analyses enable AD risk prediction well before disease onset, which is critical for early interventions. However, current analytical approaches have limited ability to accurately estimate the risk effects of genetic variants owing to epistatic effects, which have been overlooked in most of the previous studies, resulting in unsatisfactory disease risk prediction. Compared to existing models for estimating disease polygenic risk, deep learning methods can more accurately model the nonlinear grouping effects among the disease-associated variants. Thus, we aim to model the AD risk prediction using deep learning methods.

Methods: We modeled AD polygenic risk by applying the deep learning methods to the genetic data from existing AD cohorts (n = 11,352). Meanwhile, we also compared the accuracy of this result with the AD risk prediction results obtained from the genetic prediction models including weighted polygenic risk score and Least Absolute Shrinkage and Selection Operator (LASSO) methods.

Results: Our results suggest that the deep learning methods outperformed existing models (i.e., weighted polygenic risk score and LASSO models) for classifying disease risk.

Conclusions: Our results demonstrate the utility of deep learning methods for modeling the genetic risks of human diseases, which can facilitate both disease risk classification and the study of disease mechanisms.
Aims: Genetic studies reveal that single-nucleotide polymorphisms (SNPs) of SPI1 are associated with Alzheimer’s disease (AD). Particularly, rs1057233, which tags a common haplotype, is associated with decreased AD risk and SPI1 expression in myeloid cells. However, the association of SPI1 SNPs and haplotypes with AD in the Chinese population remains unclear. We aimed to examine the association of SPI1 SNPs and haplotypes with AD in the Chinese population, and investigate their underlying mechanism in modulating AD risk.

Methods: We conducted genetic analysis of 3 SPI1 SNPs (rs1057233, rs3740688, and rs78245530) and their haplotypes in a Chinese cohort (n = 333 patients with AD, n = 721 healthy controls). We also probed public European-descent AD cohorts and gene expression datasets to investigate the putative function of the identified haplotypes.

Results: We demonstrated SPI1 SNP rs3740688 (odds ratio = 0.72 [0.58-0.89]) was significantly associated with decreased AD risk in the Chinese population and identified 2 AD-protective haplotypes [haplotype β (tagged by rs3740688 and rs1057233) and γ (tagged by rs3740688 and rs78245530)]. Haplotype β and γ are associated with decreased SPI1 gene expression in the blood and brain tissues, respectively. The regulatory roles of the identified haplotypes are suggested to be associated with the changes of miRNA binding and the epigenetic landscape. Our results suggest these AD-protective SPI1 haplotypes regulate pathways involved in immune and neuronal functions.

Conclusions: This study firstly reports the association of SPI1 with AD in the Chinese population and identifies SPI1 haplotypes that are associated with decreased AD risk and SPI1 gene expression.
POSTERS

GENETIC MODIFIERS OF ALZHEIMER’S DISEASE

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Aims: Alzheimer`s disease (AD) is one of the most predominant neurodegenerative diseases. Only a small fraction of cases can be explained by a single gene mutation, suggesting that multiple factors could be at play in AD progression. To address this question we investigated the role of ADAM17, which has been recently associated with a familial form of AD, within the gene regulatory network leading to AD.

Methods: To model AD, we studied cholinergic neurons generated from human induced pluripotent stem cells (iPSC), and characterized them at single cell resolution by RNA sequencing and imaging.

Results: In-depth analysis of single cell transcriptomic readout provides insight into gene regulatory interactions involved in AD progression. The preliminary analysis indicates also impairment of Ca²⁺ homeostasis.

Conclusions: By combining this multi-pronged approach, we are able to link possible mutation-specific to cellular processes and explore compensating mechanisms preventing early age AD onset.
ASSOCIATION BETWEEN DIET QUALITY OR MEDITERRANEAN DIET IN MID-LIFE AND DEMENTIA INCIDENCE OVER A 20-YEAR PERIOD

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Aims: Our aim was to investigate whether adherence to western conventional dietary recommendations or to a Mediterranean diet are associated with subsequent lower risk of developing all-cause dementia, Alzheimer’s disease (AD), vascular dementia (VaD), or to future accumulation of β-amyloid (Aβ).

Methods: Baseline examination in the Swedish population-based Malmö Diet and Cancer Study (MDCS) took place in 1991-1996. Participants with complete baseline dietary examination (7-day food diary, detailed food frequency questionnaire and one-hour interview) were included in the study (n=28,025). Cerebrospinal fluid (CSF) Aβ42 was available in a sub-population (n=738). Cox proportional hazard models were used to examine associations between diet and risk of developing dementia.

Results: During a median of 19.8 years of follow-up, 1,943 (6.9%) participants were diagnosed with dementia. Individuals adhering to dietary recommendations did not have lower risk of developing all-cause dementia (HR 0.98, 95% CI 0.95-1.02), AD (HR 0.99, 95% CI 0.95-1.04) or VaD (HR 0.98, 95% CI 0.91-1.06). Neither did adherence to Mediterranean diet lower the risk of developing all-cause dementia (HR 0.93 95% CI 0.75-1.15), AD (HR 0.90, 95% CI 0.68-1.19) or VaD (HR 1.00, 95% CI 0.65-1.55). No significant association was found between higher diet quality index score or Mediterranean diet score and abnormal Aβ accumulation (OR 1.14, 95% CI 0.99-1.31) and (OR 0.98, 95% CI 0.85-1.14), respectively.

Conclusions: In this long-term prospective cohort study, adherence to dietary recommendations or to the Mediterranean diet were not significantly associated with subsequent reduced risk for developing all-cause dementia, AD or VaD.
COMPLEX GENE–ENVIRONMENT INTERACTIONS IN LATE-ONSET ALZHEIMER’S DISEASE: THE EFFECT MODIFICATION BY PILRA POLYMORPHISM ON APOE4, GM17, AND HERPES SIMPLEX VIRUS TYPE 1

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Aims: PILRA (homozygote rs1859788 A>G) has been suggested to be a protective variant for Alzheimer’s disease (AD) and is an entry co-receptor for herpes simplex virus-1.

Methods: We conducted a nested case-control study of 360 1:1-matched AD subjects. Interactions between PILRA, APOE, and GM 17 for AD risk were modelled. A composite variable for carriage of APOE risk variants (ε3/ε4, ε4/ε4) or GM 17/17 was used to evaluate their combined effect. The associations were cross-validated using two independent whole-genome sequencing datasets: a family-based AD sample from the National Institute of Mental Health (NIMH) and an AD-case control sample from National Institute of Aging (NIA) Alzheimer’s disease Sequencing Project (ADSP).

Results: We found negative interactions between PILRA A/A and GM 17/17 (OR 0.17, 95 % CI 0.03-0.87) and between PILRA A/A and the composite variable of APOE or GM for AD risk (OR 0.12, 95 % CI 0.03-0.51). A joint effect of PILRA and PILRA A/A × GM 17/17 for AD was observed (p .02).

Conclusions: Here, we report a negative effect modification by PILRA on APOE and GM 17 high-risk variants for future AD risk in two independent datasets. This highlights the complex genetics of AD.
Aims: Our aims are to identify and characterize microglial subpopulations in the Alzheimer's disease human brain.

Methods: We took advantage of publicly available single-nucleus RNA sequencing (snRNAseq) data from human brains with different pathological burdens classified as Braak stages 0, 2 and 6 (which represent the cortical-free, early and late stages of AD, respectively).

Results: Using Seurat, we identified 13 microglial clusters based on gene expression. While the cluster 2 was predominant in the Braak stage 0, the clusters 4 and 5 predominate in Braak stage 2 and the other clusters predominate in the Braak stage 6. Gene set enrichment analyses using the top markers of those clusters showed an enrichment for terms related with cellular respiration for cluster 2 (Braak Stage 0), enzymatic activity, protein metabolism and cell differentiation for clusters 4 and 5 (Braak stage 2) and immune-inflammatory response for the other clusters (Braak stage 6). Using Monocle, we reconstructed the trajectories of these clusters starting from cluster 2 and found three independent trajectories, suggesting that different microglial subpopulations could be generated by separate mechanisms. Lastly, we analyzed the expression of genes associated with disease-associate microglia (DAM) phenotype. The DAM stage 1 markers were highly expressed in clusters 3, 4 and 5, whereas markers of DAM stage 2 were highly expressed in the clusters 6, 7, 11 and 12.

Conclusions: Altogether, our results indicate that snRNAseq allows the identification of microglial subpopulations, including DAM, in the AD brain.
AD KNOWLEDGE PORTAL AND AGORA: RESOURCES THAT EMPOWER THE RESEARCH COMMUNITY TO FIND, ACCESS, AND RE-USE DATA AND TOOLS RELATED TO DEMENTIA AND AGING

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Aims: To identify therapies and drug targets for Alzheimer’s disease (AD), it is necessary to assess impact of cellular and molecular dysfunction on disease etiology. The AD Knowledge Portal (https://adknowledgeportal.org) and Agora (https://agora.adknowledgeportal.org) are community-driven resources developed to support researchers as they: generate hypotheses about molecular mechanisms, evaluate hypotheses via independent experiments, and prioritize new mechanisms for therapeutic development. The Portal and Agora are open access tools maximizing therapeutic discovery through re-use of data from a large range of biospecimens and individuals, and analytical results for putative drug targets.

Methods: Created to support research outputs from the NIA funded Accelerating Medicines Partnership-Alzheimer’s Disease, the Portal now supports 55 grants related to dementia and aging. Generated from central and peripheral measurements from brain banks, longitudinal cohorts, and model systems, available data spans assays from genomic variants to imaging, cognitive assessments, and more. Model systems represented in the Portal include iPSC-derived cell types and organoids, and novel, unlimited-use Late Onset AD mouse models.

Results: The Portal features a cloud-based analytical workspace providing access to preconfigured computational resources to work with data. Agora, a visual results explorer compiling evidence for gene association with AD, presents a subset of the processed data, results of ‘omics analyses generated from Portal data, a list of nominated targets and includes a catalogue of results from targeted validation studies.

Conclusions: The Portal and Agora offer the research community an accessible source of data, tools, and results for the study of AD and related dementias.
RELEVANCE OF RHBDL4-MEDIATED APP PROCESSING FOR ALZHEIMER’S DISEASE

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Aims: Autosomal dominant inherited mutations causatively link the amyloid precursor protein (APP) to Alzheimer’s disease (AD) and one of its proteolytic cleavage products, Aβ peptides, is a hallmark of AD. While APP’s relevance in the pathogenesis of the disease is clear, its physiological functions remain poorly characterized. Unveiling APP’s physiological functions and defining the triggers leading to Aβ production are crucial to determining the cellular conditions underlying AD. We have discovered a non-canonical processing pathway of APP mediated by the rhomboid protease RHBDL4. We have shown in vitro that RHBDL4 cleavage resulted in novel APP fragments, modifying APP cell surface levels as well as Aβ production. Here, we aim to determine the physiological relevance of this novel pathway in the context of AD.

Methods: We crossed two mouse models harboring familial APP mutations (McGill-Thy1-hAPP and J20) with RHBDL4 null mice. Memory performance is determined through Y maze and NOR tests on 5-month-old mice. APP and Aβ levels are measured from brain lysates by western blot and ELISA.

Results: We showed that the memory deficit observed in McGill-Thy1-hAPP mice at 5 months of age was rescued by the absence of RHBDL4, likely attributing to its effects on APP and Aβ levels. Using a more robust model for amyloidosis, we aim to corroborate the physiological impact of RHBDL4 on APP processing and memory in J20 mice lacking RHBDL4.

Conclusions: This study asserts RHBDL4’s importance for APP physiology in vivo, thus allowing the use of RHBDL4 as a new tool to further our knowledge on APP’s functions.
THE CTALPHA16 DOMAIN OF THE SECRETED APP ECTODomain RESCUES SYNAPTIC IMPAIRMENTS IN VIVO

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Aims: We recently showed that secreted APPsalpa, but not APPsbeta, prominently rescues spine density deficits of NexCre cDKO mice, a conditional double knockout for APP and APLP2. Further, we showed that a synthetic peptide encompassing the C-terminal 16 amino acids of APPsalpa facilitates LTP to the same extent as APPsalpa and may thus constitute the minimal functional domain of APPsalpa. Here, we studied the potential of this peptide to ameliorate synaptic deficits in vivo.

Methods: To this end we designed an AAV9 vector to express CTalpha16 in vivo in the hippocampus of conditional double knockout animals.

Results: As short peptides are difficult to express, we employed a system previously used to express Abeta in transgenic mice. To this end, CTalpha16 was fused to the pre-pro-sequence of murine thyrotropin-releasing hormone (mTRH) to direct the precursor to the ER and subsequent cleavage within the secretory pathway by prohormone convertases. Indeed, stereotactic injection of AAV-CTalpha16 into the hippocampus lead to highly efficient expression of HA-tagged CTalpha16. We could further show that CTalpha16 can be expressed at the same level as APPsalpa and is properly processed and secreted. Upon intracranial injection of AAV9-CTalpha16 into the hippocampus of NexCre cDKO mice we found that spine density deficits could be completely rescued and even exceeded that of littermate controls. In ongoing experiments, we are assessing whether AAV-CTalpha16 may also facilitate LTP and improve learning and memory.

Conclusions: Our results point towards a prominent synaptotrophic function of CTalpha16 in vivo that may be of interest for future therapeutic applications.
REVEALING HIDDEN SENSORIMOTOR MEMORIES IN MICE WITH AD-RELEVANT PATHOLOGY

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**Aims:** Alzheimer’s disease (AD) results in a slow deterioration of cognitive capacities due to neurodegeneration. Interestingly, AD patients can exhibit cognitive fluctuations and, contextual factors are critical for them to be able to unlock their memories. Exploration of the neural basis of cognitive fluctuations has been hampered without a behavioral approach to dissociate memories from contextual-performance.

**Methods:** Our previous work demonstrated that interleaving ‘reinforced’ with non-reinforced trials in an auditory go/no-go discrimination task, allows us to do this distinction. We used this approach, with two-photon calcium imaging on AD-relevant mice (APP/PS1+), to determine whether amyloid accumulation impacts underlying sensorimotor memories and/or contextual-performance in an age dependent manner.

**Results:** Importantly, peripheral auditory function, measured with ABRs, was similar between WT and APP/PS1+ mice. We found that while contextual-performance is significantly impaired in young-adult APP/PS1+ mice, these animals show only minor impairments in the underlying sensorimotor memories. However, middle-aged APP/PS1+ mice show deficits in both domains. The impairment found in the young-adults was accompanied by a reduction in stimulus selectivity in the auditory cortex of APP/PS1+ mice, especially in reinforced trials. Ongoing analyses aim to identify whether this impairment is cortex-wide or is concentrated near Aβ plaques. Finally, these effects were recapitulated by using a reinforcement learning model that accounts for changes in contextual signals. The main network model parameters affected between the control and the APP/PS1+ mice were those governing contextual scaling and behavioral inhibition.

**Conclusions:** These results suggest that Aβ deposition impacts circuits involved in contextual computations before those involved in acquiring knowledge.
Aims: The Fe65 protein family members, comprising Fe65, Fe65L1, and Fe65L2, were identified as interacting partners of the amyloid precursor protein (APP), implicated in the pathogenesis of Alzheimer's disease. Similarities in phenotypes of APP and Fe65 family knockout (KO) mice suggest a common intracellular signaling cascade. However, the molecular mechanism is yet unclear.

Methods: We performed detailed histological analyses of dendrites and apical second ordered dendritic spines in hippocampus CA1 region of Fe65 family KO mice. Subsequently, we analyzed the actin cytoskeleton using fluorescence recovery after photobleaching (FRAP) and live-cell imaging.

Results: Akin to APP KO neurons, Fe65/Fe65L1 KOs showed reduced dendritic length and branching, leading to a reduced complexity of arborization. Further, we could demonstrate a shift in maturation of dendritic spines, both pointing towards participation of Fe65 and APP in actin regulation that was verified by FRAP analysis. Rescue experiments with deletion constructs of Fe65 highly conserved domains (WW, PTB1, PTB2) enabled us to compile a model of putative interactions and signaling pathways, how APP and Fe65 may be involved in modulating the actin cytoskeleton.

Conclusions: Together, our data elucidates the Fe65 protein family as a crucial downstream factor of APP intracellular signaling cascades, implicated in dendritic outgrowth and spine plasticity.
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Aims: Amyloid beta (Aβ) is generated by sequential proteolytic cleavage of the Amyloid Precursor Protein (APP) by different secretases that have distinct subcellular localizations. Thus, APP trafficking and duration of stay in individual compartments of the secretory endocytic pathway strongly affects its processing. In particular endocytosis of APP is an important step in this context, as BACE 1 activity is mostly present in endosomes. In previous studies we could show that APP endocytosis is regulated by different motifs, including the classical NPTY endocytosis motif and the basolateral sorting signal (BaSS), involving Rab5a activating GDP/GTP exchange factor RME6. However, it remained unclear by which molecular processes it is decided whether APP is taken up and which consequences are to be expected for processing.

Methods: We performed antibody uptake assays with transfected neuronal cells to analyze the internalization rate of myc tagged APP or endocytosis deficient APP mutants. The amount of endocytosed protein was determined by measuring the intensity of surface protein compared to internalized protein. Moreover, the processing products of APP and APP mutants were analyzed.

Results: We generated different APP mutants and determined their endocytosis rate. Interestingly, we found in addition to the established intracellular endocytosis motifs, like NPTY or BaSS, that also extracellular domains/motifs of APP affect its endocytosis. The influence of these extracellular domains/motifs on endocytosis and processing of APP were characterized in more detail.

Conclusions: Together, our data will help to get a better understanding of the molecular mechanisms underlying APP endocytosis, opening the avenue to novel therapeutic targets against AD.
ACTIVATION OF A CK2/MAPK KINASE PATHWAY BY THE AMYLOID PRECURSOR PROTEIN INTRACELLULAR DOMAIN (AICD) AFFECTS AXONAL TRANSPORT CHARACTERISTICS

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Aims: Most adult-onset neurodegenerative diseases including Alzheimer’s Disease (AD) show deficits in axonal transport (AT). Interestingly, AT-deficits induced by AD-related mutations in the Amyloid Precursor Protein (APP)-processing secretase presenilin-1 as well as by oligomers of Amyloid-beta peptides derived from APP-cleavage were associated with kinase activation modulating AT. This project aimed to investigate the still unknown function of the cytosolic APP intracellular domain (AICD), released by gamma-secretase-cleavage, on AT.

Methods: To test for potential effects of the AICD on AT of specific vesicles, we performed live cell imaging of mouse neurons expressing fluorescently-labelled forms of full-length APP (APP-FL) or APP-deletion mutants as well as other transport cargos in combination with AICD co-expression. Further, to investigate directly AICD-induced effects on AT, vesicle motility assays on isolated squid axoplasm preparations were performed.

Results: APP lacking the NPTY-motif displayed increased AT-rates, compared to APP-FL. Furthermore, overexpression of the AICD peptide changed the AT-characteristics of different APP-unrelated cargo proteins. In line with this, the total vesicle movement in squid axoplasm was reduced after AICD-perfusion. Mapping of the essential domain revealed that mostly the NPTY-motif was responsible for this effect. Further, AICD-incubation caused an increased phosphorylation of kinesin-1 heavy and light chain subunits and inhibitors targeting casein kinase 2 (CK2) and the MAPK pathway were capable to rescue APP-mediated AT-impairment.

Conclusions: Our results revealed that AICD can increase kinase activity, affecting kinesin-1 phosphorylation that in turn affects anterograde AT. This clearly indicates that packaging of APP in AT-vesicles and/or alterations in AICD production might contribute to AT-deficits observed in AD.
Aims: The amyloid precursor protein (APP) and its homologues APLP1 and APLP2 are type I transmembrane proteins with a large ectodomain that is further subdivided into the E1 and E2 domain. APP/APLPs bind to copper or zinc ions, as well as heparin, and have been reported to play a function in synapse formation and plasticity, likely mediated via trans-cellular dimerization in a homo- or heterotypic manner. However, other synaptic functions of APP depend on secreted APP fragments. The major aim of this work was to identify and characterize synaptic factors that affect the stability of APP/APLP dimers.

Methods: Mostly, a bead aggregation assay (BAA) was used. Thereby, APP/APLP was coupled to beads, which were incubated in various conditions. In case of trans-directed interaction, beads formed clusters. Furthermore, liquid chromatography–mass spectrometry was used to identify APP-interacting proteins.

Results: Astrocyte conditioned medium caused a stabilization of APP dimerization in BAAs. Subsequent mass spectrometric analysis of APP-associated proteins revealed 72 astrocyte-secreted proteins, including several heparan sulfate proteoglycans (HSPGs), reported to interact with APP. Interestingly, APP dimerization was not affected by any of the tested HSPGs, including glypicans, syndecan and agrin. However, one highly promising protein candidate, known to promote synapse formation, stabilized APP dimerization and is currently analyzed in regard to a functional interaction with APP/APLPs.

Conclusions: Together, we identified a synaptogenic protein secreted by astrocytes that favours APP trans-directed dimerization, indicating that astrocytes might control stabilization of APP trans-cellular dimers at the synapse, having important consequences for APP physiological as well as pathological properties.
Aims: Biological membranes demonstrate lateral and transverse asymmetric lipid distribution, and these asymmetries induce versatile membrane biophysical properties, affecting several physiological and pathological processes. This includes the processing of amyloid precursor protein (APP), a single-pass transmembrane protein, whose main proteolytic fragment, amyloid-beta has been implicated in Alzheimer’s disease (AD) pathogenesis. Our research aimed at solubilizing and purifying membrane proteins within their native lipid environment into nanodiscs, using styrene-maleic acid (SMA) and diisobutylene maleic acid (DIBMA) copolymers.

Methods: Rather than using traditional methods, we solubilized HeLa cells using detergent-free SMA and DIBMA, to generate the so called SMA and DIBMA lipid particles (SMALPs, DIBMALPs). These SMALPs and DIBMALPs were subjected to immunoisolation, and further evaluated by proteomic and lipidomic analyses.

Results: Our data could show that both SMA and DIBMA exhibited distinct solubilization characteristics. Detailed mass spectrometric analysis of enriched SMALPs and DIBMALPs further revealed that solubilization kinetics varied between diverse subcellular compartments and protein complexes. Moreover, we could establish an efficient immunoisolation protocol for APP containing DIBMALPs that allowed detailed proteomic and lipidomic analyses of the native APP membrane environment.

Conclusions: DIBMA, as well as SMA, quantitatively solubilized membrane proteins from human cells, and preserved membrane protein complexes. In combination with lipidomic and proteomic approaches, we will have the opportunity to characterize in more detail how membrane proteins behave in pathological conditions, such as in AD.
Aims: APP has is expressed by ciliated cells. In the present project we therefore aimed at investigating the presence of APP in the cilium of othic, olfactory and ependymal cells. The cilium are important mediators of sensory input or to sense ques in and promote CSF flow.

Methods: We have used zebrafish, mouse and human samples to identify App distribution within the cilium of ciliated cells using in situ hybridization to and immunohistochemical staining. We used high and super-resolution confocal imaging to co-localize App with cilia markers in the cilium and to count and measure the morphology of individual cilia. To address the impact of App on the cilium we knocked out the zebrafish App genes, appa and appb, using the CRISPR/Cas9 technology. CSF movement was addressed by tracking fluorescent nano-beads injected into the brain ventricles of larval zebrafish.

Results: We report that Appa and Appb are expressed by ciliated cells and become localized at the membrane of cilia in the olfactory epithelium, otic vesicle and in the brain ventricles of zebrafish. App in ependymal cilia persisted in adult zebrafish and was also detected in mouse and human brain. Finally, we found morphologically abnormal ependymal cilia and smaller brain ventricles in appa−/− appb−/− mutant zebrafish. Preliminary data indicate changes in CSF flow of the double mutant appa−/− appb−/− zebrafish during development.

Conclusions: Our findings demonstrate an evolutionary conserved localisation of APP to cilia and suggest a role of App in ciliogenesis and cilia-related functions.
Aims: Studies on genetic robustness recently revealed mechanisms by which an organism can mask genetic mutations through activation of homologous genes, known as transcriptional adaptation (TA). In this study we show that genetic mutations, which introduce premature stop codons (PTC) in the app genes, activate TA of other app family members in zebrafish.

Methods: CRISPR/Cas9 method was used to generate zebrafish mutants without appb promoter part (RNA-less mutants) together with different molecular techniques such as quantitative PCR to measure the RNA levels.

Results: We found TA by appb to be dose-dependent and to activate expression of the closely related gene, appa faster than the less homologous aplp2 gene. In contrast to appa and aplp2, aplp1 was regulated in a more complex manner that seems to be dependent on the presence of wildtype appb. Here we show that the transcriptional response requires the presence of mutant mRNA and does not depend on protein level or translation. In addition, we did not observe TA by app in adult zebrafish brain nor in vitro in a human neuroblastoma cell line. However, the lack of Mauthner cell (M-cell) phenotype in the mRNA-less mutants indicates that TA by appa and aplp2 is not enough to explain differences observed between appb mutants and the morpholino-mediated knockdown but might rather be the result of aplp1 expression.

Conclusions: Our results show that mutations in app genes induce compensation between app family members through TA in zebrafish that potentially could mask the true function of App.
Aims: Amyloid beta (Aβ) is part of senile plaques in brains affected by Alzheimer’s disease. Different Aβ aggregation states bind various receptors, for example TREM2. TREM2 is a receptor expressed by microglia, the brain immune cells. Here, we analyzed the microglial response to Aβ-aggregates and its relation to TREM2.

Methods: 10 µM or 50 µM of recombinant Aβ1-42 (recAβ, rPeptides) and of scrambled Aβ1-42 (scrAβ, random amino acid sequence) were prepared according to the manufacture’s protocol in 10xPBS or low salt 10xPBS. The aggregation was monitored with a thioflavine T assay. Aggregated Aβ species (0.3 µM) were supplemented to N9 wild-type (N9-wt) and to N9 Trem2 knock-out cells (N9-ko). The nitrite level in the medium was measured with a 2,3-diaminonaphthalene assay 48 hours later.

Results: 50 µM recAβ (4 hours) in 10xPBS reached the aggregation peak faster than 10 µM (51 hours). Low salt 10xPBS increased the aggregation time (50 µM=41 hours, 10 µM=100 hours). ScrAβ neither aggregated nor triggered N9 cell nitrite release. However, recAβ-supplementation resulted in a higher nitrite response of N9-wt than N9-ko cells. Furthermore, 10 µM recAβ activated exclusively N9-wt cells with a low nitrite level.

Conclusions: The correct Aβ sequence is necessary for the aggregation and for a microglia response. Furthermore, a combination of low recAβ concentration in a low salt buffer delayed and changed the fibrillation process. An Aβ species mixture was produced, which strongly binds the TREM2 receptor on microglia cells with a lower inflammatory response than the higher concentrated recAβ species. Further analysis are ongoing.
ISOFORM-SPECIFIC EFFECTS OF APOLIPOPROTEIN E ON HYDROGEN PEROXIDE-INDUCED APOPTOSIS IN HUMAN INDUCED PLURIPOTENT STEM CELL (IPSC)-DERIVED CORTICAL NEURONS

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Aims: The Apolipoprotein E gene (APOE) has been identified as the strongest genetic risk factor for late-onset Alzheimer’s disease (AD), and the coded ApoE protein possesses isoform-specific antioxidant activity. Hydrogen peroxide (H₂O₂)-induced neuronal apoptosis is critical to the pathology of neurodegenerative diseases, such as AD. Whether ApoE could protect neurons from apoptosis in an isoform-specific manner remains controversial. Here, we aim to dissect the neuroprotective function of ApoE in human neuronal culture.

Methods: We derived human iPSCs to cortical neurons, and H₂O₂ treatment was employed to induce neuronal apoptosis. Three ApoE isoforms were examined.

Results: We found that ApoE2 and ApoE3 pretreatments significantly attenuated neuronal apoptosis through binding ApoE receptor(s). However, ApoE4 showed no neuroprotection and higher concentrations of ApoE4 even displayed toxic effect. We further identified that ApoE2 and ApoE3 upregulated Akt activity and inhibited FoxO3a transcriptional activity in the presence of H₂O₂, resulting in decreased expression level of downstream Bim.

Conclusions: We propose that ApoE2 and ApoE3 alleviate H₂O₂-induced apoptosis in human iPSC-derived neuronal culture via regulating Akt/FoxO3a/Bim signaling pathway, whereas ApoE4 has no protective effect. These results provide an alternative mechanistic explanation on how ApoE isoforms influence the risk of AD onset as well as a promising therapeutic target for diseases related to neuronal apoptosis in the central nervous system.
THE CELLULAR AND SYNAPTIC ROLE OF APOE IN MODELS OF ALZHEIMER’S DISEASE

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Aims: Apolipoprotein E4 (ApoE4), the major genetic risk factor for Alzheimer’s disease (AD), has been described to play a role in many AD-related processes and cell types, however the most critical mechanism(s) and source of ApoE in AD remains unclear. Changes in endosomes and synapses are early alterations in neurons related to AD. ApoE4 has been associated with endosomal abnormalities, such as endosome enlargement and impaired endosomal recycling, and dysregulated synaptic plasticity. Here, we study the effects of different ApoE isoforms on cellular and synaptic changes in AD and non-AD conditions.

Methods: Astrocyte-conditioned medium from ApoE KO, ApoE3-KI and ApoE4-KI mice and recombinant ApoE are used as sources for human ApoE. Neuroblastoma cells and primary neurons, either endogenously expressing or treated with exogenous ApoEs, are analyzed by immunofluorescence, Western blot and live cell calcium imaging.

Results: Human ApoEs localize to synaptic terminals. A cell-source specific effect of ApoE on neuronal activity is observed, whereby astrocyte- and neuron-produced ApoE differentially influence neuronal excitability. In addition to a synapse-associated localization, a subset of ApoE appears to co-localize to LAMP1-positive vesicles. In an AD cell model, ApoE and APP metabolites are shown to co-localize sub-cellularly.

Conclusions: ApoE appears to have a synaptic and endosomal localization in neurons and neuron-like cells and influences neuronal activity in an ApoE isoform and cell source specific manner. Determining the neurobiology of ApoE in connection with cellular sites vulnerable to early AD changes can contribute to a better understanding of the role of ApoE in AD.
ISOFORM-SPECIFIC BINDING OF APOE TO COMPLEMENT REGULATOR FACTOR H REDUCES AMYLOID BETA-INDUCED NEUROINFLAMMATION

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Aims: Complement-mediated neuroinflammation plays a crucial role in Alzheimer’s disease (AD). Apolipoprotein E (apoE) is a pivotal molecule involved in amyloid beta (Abeta) clearance, in particular apoE4 is considered the strongest genetic risk factor for AD. ApoE interacts with complement regulator factor H (FH) but the extent of this interaction has not been studied yet. Our aim is to elucidate whether this interaction has a role in neuroinflammation.

Methods: Protein interactions were measured by microscale thermophoresis. Flow cytometry was used for phagocytosis and CR3 binding assays. Formation and stoichiometry of FH/apoE/Abeta1-42 complexes were measured by western blotting and single-molecule fluorescence imaging. Cell responses were analyzed using mRNA sequencing. Immunohistochemistry was used to image apoE, Abeta, FH and C1q in vivo in human cortical biopsy samples.

Results: Flow cytometry and transcriptomic analysis revealed that apoE and FH reduce binding of Abeta1-42 to the phagocytic receptor CR3 and phagocytosis by microglia which alter the expression of genes involved in AD. Moreover, FH forms stable complement-resistant apoE isoform-specific oligomers with apoE/Abeta1-42 complexes (apoE2>apoE4), it reduces Abeta1-42 oligomerization and competes for the binding of complement activator C1q to apoE. The in vivo data on colocalization of apoE and FH near Abeta plaques in the brain provided evidence for the protective role of this interaction in Abeta-induced neuroinflammation.

Conclusions: Here we demonstrate that isoform-specific binding of apoE to factor H reduces Abeta-induced neuroinflammation, thereby suggesting a novel mechanism on how the strongest known genetic risk factor for AD predisposes to neuroinflammation.
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Aims: Long amyloid beta peptides may act as substrates or products in the gamma-secretase-mediated proteolysis. We tested the hypothesis that Abeta peptides, when present at high concentrations (as in the Alzheimer’s disease brains), bind to gamma-secretase and inhibit the processing of gamma-secretase substrates with consequent dysregulation of downstream cellular signalling.

Methods: To test the hypothesis, we conducted rigorous kinetic analyses of gamma-secretase activity in cell-free systems in the presence of a series of Abeta peptides. Western blotting and mass spectrometry were used to define enzyme activity against a number of substrates. We also conducted cell-based assays to assess in living cells the impact of Abeta on the levels of unprocessed substrates and NOTCH signalling.

Results: Kinetic evaluation of gamma-secretase activity in cell-free conditions demonstrated that Abeta1-42 exerted feedback inhibition, affecting proteolysis of all tested substrates (NOTCH, ERBB4 and neurexin). The studies also gave evidence for the importance for inhibition of the N-terminal domain of Abeta: neither the human 17-42 peptide (i.e.,p3) nor mouse/rat Abeta1-42 (differing from human only by three amino acids in the N-terminal domain) exerted inhibition. The pathophysiological relevance of the observations was supported by the accumulation of gamma-secretase substrates and disruption of cell signalling cascades mediated by NOTCH cleavage.

Conclusions: That Abeta1-42 reduced processing of several gamma-secretase substrates, demonstrated that inhibition was not substrate selective. Feedback inhibition by pathologically relevant Abeta peptides of the gamma-secretase activity suggests a novel mechanism by which Abeta1-42 contributes to AD pathogenesis.
NEW TOOLS TO EXPLORE THE BIOLOGICAL FUNCTION OF ETA-SECRETASE-DERIVED APP-C-TERMINAL FRAGMENT

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**Aims:** Amyloid precursor protein (APP) is a transmembrane protein undergoing canonical cleavages by alpha, beta and gamma-secretases. Recently, the matrix metalloprotease MT5-MMP, referred to as eta-secretase, has been identified as a novel APP cleaving enzyme (Baranger et al., 2016). This enzyme produces a transmembrane eta-CTF that undergoes subsequent cleavages producing A eta alpha and A eta beta fragments (Willem et al., 2015).

**Methods:** In this study, we first characterized a novel antibody directed against an N-terminal epitope of eta-CTF (eta-CTF-N Ter), making it specific for eta-CTF, A eta alpha and A eta beta. This antibody labels native and denatured eta-CTF and is useful for immunobLOTS, immunohistochemistry and recognizes both murine and human species. Of most interest, this novel antibody displays exclusive selectivity towards eta-CTF vs C99 or C83 fragments.

**Results:** This immunological probe was used to examine the fate of eta-CTF in SH-SY5Y. By a pharmacological approach, we confirm that eta-CTF is processed by alpha, beta and gamma-secretases. Further, we show that eta-CTF is eliminated by both proteasomal and autophagic degradation. Finally, we performed immunocytochemistry on HeLa cells overexpressing eta-CTF and immunostaining on organotypic or paraffin brain slices of mice infected with AAV-Eta-CTF.

**Conclusions:** Our results demonstrate the usefulness of a novel immunological probe for both biochemical and in situ analyses of eta-CTF, in mice and human models.
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POSTERS

CRACKING THE MYSTERIOUS CODE OF GAMMA-SECRETASE SUBSTRATE RECOGNITION BY COMBINING COMPARATIVE PHYSICOCHEMICAL PROFILING AND EXPLAINABLE ARTIFICIAL INTELLIGENCE

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Aims: Despite two decades of intense research, it has remained a mystery how the Alzheimer’s disease associated gamma-secretase recognizes its substrates, which are cleaved within their transmembrane domain (TMD). Our main question is whether there is a common property defining gamma-secretase substrates. We hypothesized that this property is a set of various features, which we define as physicochemical properties (e.g., volume or charge) present at distinct segments or patterns within the substrate sequence.

Methods: To test our hypothesis, we developed a unique bioinformatic method named Comparative Physicochemical Profiling (CPP). CPP aims to identify a collection of features that are most discriminant between two sets of protein sequences. Machine learning models were trained to select the best features and to predict de novo gamma-secretase substrates and non-substrates. To elucidate the prediction results, we combined CPP with SHAP (Shapley Additive exPlanations), which is an explainable artificial intelligence tool. Finally, predicted substrates were biochemically tested by independent cleavage assays.

Results: Machine learning models achieved an accuracy of >85% for gamma-secretase substrate prediction. In an ongoing analysis, several predicted substrates could be validated. Additionally, we identified specific substrate features like a higher beta-sheet tendency within the C-terminal TMD or a well-defined TMD anchor.

Conclusions: Our work provides a toolset to predict gamma-secretase substrates by obtaining distinct physicochemical profiles. We propose a novel cleavage mechanism named unfolding-floating-ball model, which highlights (a) the importance of the TMD anchor stabilizing substrates at the membrane surface and (b) the higher beta-sheet tendency facilitating helical unfolding and, thus, cleavage.
METADYNAMICS SIMULATIONS OF GAMMA-SECRETASES BOUND TO BETA-AMYLOID PEPTIDES REVEAL THE PREFERENCE OF PRESENILIN-2 FOR BETA-AMYLOID-42 PRODUCTION.

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Aims: The generation of β-amyloid proteins (Aβ), a function of gamma-secretase processing of Amyloid Precursor Protein (APP), is a potential therapeutic target to treat Alzheimer’s disease. A viable strategy for therapeutic targeting of gamma-secretase is to design molecules that reduce the Aβ42:Aβ40 ratio. While such modulators of gamma-secretase have been identified, the discovery and optimisation of further modulator leads has been hampered by a limited understanding of the structure and dynamics of gamma-secretase, as well as a limited understanding of how the presenilin (PS) isoforms (PS1 and PS2), the catalytic subunit of gamma-secretase, influence product generation.

Methods: Molecular models of PS1- and PS2-gamma-secretase bound to APP-derived substrates associated with Aβ40 generation (Aβ49, Aβ46, Aβ43, Aβ40) and Aβ42 generation (Aβ48, Aβ45, Aβ42, Aβ38) were built and metadynamics simulation used to broadly sample the conformational ensembles of the complexes. Binding energy calculations were performed on identified low energy states.

Results: PS1-containing gamma-secretase exhibits a broader low energy conformational ensemble when bound to Aβ49, while the PS2-containing complex exhibits a broader low energy conformational ensemble when bound to Aβ48. Computed binding energies suggest that the PS1-gamma-secretase binds Aβ42 ‘tighter’, which may increase the likelihood of Aβ38 production, while the PS2-Aβ42 complex is significantly less stable, likely resulting in premature release of Aβ42. These results recapitulate cell-based studies and implicate PS2 containing gamma-secretase complexes in preferentially generating Aβ42.

Conclusions: Our results demonstrate the utility of computational approaches in exploring gamma-secretase structure and dynamics, and may provide a pathway for the structure-based rational design of gamma-secretase modulators.
GPR27-MEDIATED INCREASE IN CYTOSOLIC L-LACTATE IN 3T3 EMBRYONIC CELLS AND ASTROCYTES

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Aims: G-protein coupled receptors (GPCRs) consist of over 800 members in humans; one third of these represent targets for approved drugs. Here we investigated GPR27, an orphan GPCR belonging to the family of super conserved receptor genes expressed in the brain (SREB), with unknown function.

Methods: Cytosolic levels of L-lactate ([lactate]), the end-product of aerobic glycolysis, were measured by the fluorescence resonance energy transfer (FRET)-based nanosensor Laconic in single 3T3 embryonic cells and primary cultured rat astrocytes upon application of 8353, a surrogate agonist of GPR27. CRISPR-Cas9 was used to generate 3T3 cells with knocked-out (KO) GPR27 (GPR27KO3T3). To rescue the responses a GPR27-carrying plasmid was transfection into the GPR27KO3T3 cells.

Results: In 3T3 cells the application of 8353, a surrogate agonist, known to activate GPR27, resulted in an increase in [lactate], the end-product of aerobic glycolysis. A similar increase was recorded in astrocytes, an abundant type of neuroglial cells providing homeostatic support to neuronal networks in the central nervous system, which express glycogen and enzymes of aerobic glycolysis. The 8353-induced increase in [lactate], was reduced in GPR27KO3T3 cells in comparison to the wild type controls. However, the transfection of a GPR27-carrying plasmid into the GPR27KO3T3 cells, rescued the 8353-induced increase in [lactate].

Conclusions: These results indicate that stimulation of GPR27 enhances aerobic glycolysis and L-lactate production in 3T3 cells and astrocytes. Moreover, in the absence of GPR27, resting [lactate], was reduced in 3T3 cells in comparison to controls, further supporting the view that GPR27 regulates L-lactate homeostasis in the absence of any stimulation.
Aims: Due to the heterogeneity and complexity of pathologies, disease mechanisms for Alzheimer’s dementia are poorly understood. Advances in single cell RNAseq technologies provide the opportunity to characterize individual cells and the recent refinements of methods allow sequencing of individual cell nuclei, thereby allowing opportunities to generate single cell data from human biopsies. The integration with recently developed bioinformatics techniques allows modelling of both intracellular and intercellular interactions. We aimed to increase our understanding of such signaling changes in cellular pathology compared to healthy aging to uncover novel disease mechanisms.

Methods: Cell type specific gene expression profiles were compared between healthy aging and progressing disease employing a combination of internal and public single cell RNAseq datasets. Signaling networks mapped on top of normal healthy aging and disease were used to identify molecular pathways enriched in disease.

Results: Using intercellular and intracellular network engagement in healthy aging compared to disease state we generated mechanistic predictions of signaling pathways associated with disease, specific to individual cell types and relevant for signaling between cell types. We linked enriched pathways and their underlying genes with whole genome association studies to allow further refinement of disease relevant mechanisms.

Conclusions: Characterizing molecular and cellular interactions within and between cell types increases our understanding of the mechanisms underlying healthy aging and the changes occurring during progression of Alzheimer’s disease. Identification of the molecular underpinnings of the pathology of Alzheimer's disease will ultimately allow design of novel therapeutics.
POSTERS

L-SERINE PHOSPHORYLATED PATHWAY AND SERINE METABOLISM IN ALZHEIMER’S DISEASE

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Aims: Beyond ATP production, a major function of glycolysis in astrocytes is to support the synthesis of L-serine, an essential amino acid, critical for neurotransmission and synaptic plasticity as it is the main precursor of glycine and D-serine, two co-agonists of NMDAR. L-Serine synthesis proceeds via the diversion of glycolytic flux into the L-serine phosphoserine pathway. This pathway consists of three sequential enzymatic reactions involving 3 phosphoglycerate dehydrogenase (PHGDH), Phosphoserine aminotransferase 1 (PSAT), and Phosphoserine phosphatase (PSP). Since biochemical analyzes have not produced conclusive results on the evolution of L/D-serine levels in the brain of AD patients, we investigated L-serine phosphorylated pathway in AD.

Methods: Transcriptomic, proteomic, metabolomic, western blot, and HPCL analysis were performed in hippocampal samples from control and AD patients.

Results: The transcriptomic profile of AD patients did not show any variation in the expression levels of the three enzymes as well as in levels of the different transcripts identified for each gene. However, western blot and proteomic analysis highlighted that PHGDH and PSAT but not PSP levels were significantly increased in individuals affected by AD. Enantiomeric HPLC and metabolomic profile indicate that these changes were associated with a significant decrease of L-Ser levels and a significant increase of D-Ser/total-Ser ratio in AD patients. Moreover, we found that changes in serine metabolism were biological sex-dependent.

Conclusions: Given the pivotal role of astrocytic L-serine in the brain our data indicate that altered L-serine levels may contribute to damage neurotransmission and synaptic plasticity in AD patients.
METABOLIC STUDY OF SERUM, BRAIN AND PERIPHERAL ORGANS OF MODEL MOUSE APP/PS1 TO DELVE INTO THE PATHOLOGY ASSOCIATED WITH ALZHEIMER'S DISEASE

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Aims: To characterize metabolically in an comprehensive way the pathology associated with Alzheimer's disease

Methods: Direct infusion techniques in MS (DI-ESI-MS and FIA-APPI-MS) for serum analysis. Chromatographic couplings with MS (UHPLC-MS and GC-MS) for metabolomic analysis of mouse tissues: Samples: (a) Brain (hippocampus, cerebral cortex, cerebellum, striatum and olfactory bulbs) to check regional specificity of metabolic alterations and decipher neuropathological mechanisms associated with AD. (b) Peripheral organs: liver, kidney (as metabolically active organs) and spleen and thymus (organs of the immune system).

Results: The results show common disturbances in the various tissues studied in relation to the following metabolic pathways: membrane lipids, energy metabolism, oxidative stress and hyperammonemia. The most interesting results follow: Phospholipid metabolism: The metabolomic profiles showed a significant reduction in polyunsaturated fatty acid content in numerous classes of phospholipids in all samples analyzed. This decrease in PUFA-PL levels was accompanied by an increase parallel of saturated compounds, mainly derivatives of stearic acid. Sphingolipid metabolism: It was observed an increase in saturated sphingomyelins in hippocampus, and the correlative decrease in unsaturated species in the cortex brain and cerebellum. It is remarkable the reduction of long chain species (C22-C24) in the studied brain regions and the increase of these sphingolipids in serum.

Conclusions: The results show the involvement of both the central and peripheral nervous systems in the development of AD pathology in the APP/PS1 transgenic mouse. Some metabolites showed differential regulation depending on the analyzed tissue, which could be indicative of selective alterations depending on the investigated organ.
TRANSCRIPTOME-BASED BRAIN MOLECULAR PROPERTIES UNDERLYING REGIONAL VULNERABILITY TO ALZHEIMER’S DISEASE BETA-AMYLOID PATHOLOGY PROGRESSION

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Aims: Characterize molecular properties underlying regionally selective vulnerability to the spread of AD Aβ pathology, by studying the pathology progression time to different clinical disease stages estimated from longitudinal neuroimaging in relation to the transcriptional architecture of the human brain as revealed by brain-wide regional gene expression profiling from the Allen Human Brain Atlas AHBA.

Methods: The study cohort included 615 individuals (215 cognitively unimpaired(CU) Aβ−, 33 CU Aβ− converters, 103 CU Aβ+, 216 Aβ+ mild cognitively impaired(MCI), and 48 Aβ+ dementia) with longitudinal AV45 Aβ-PET from ADNI. Longitudinal AV45 data was modeled to create regional Aβ progression models. Progression time gap between clinical-disease stages (Gap1: CU Aβ+ to MCI Aβ+; Gap2 MCI Aβ+ to AD Aβ+) were estimated for each brain region. Aβ progression time gap profiles were then correlated with mRNA expression data from 21 genes associated with AD risk from AHBA.

Results: The correlations between disease progression and mRNA expression revealed two general patterns across the six genes that were marginally significant. SORL1, CD2AP and APP may play roles in causing vulnerability to amyloid accumulation, given that higher expression was correlated with shorter time to the next disease state. Conversely, CR1, PLEKHC1, and PSEN2 may have an opposite, protective effect, given that high expression of these genes was correlated with longer time to the next disease state.

Conclusions: Our findings could reveal gene expression profiles correlated with longer(protective) or
shorter (vulnerable) disease progression time, leading to potential new avenues for treatment through the up- or down-regulation of gene expression.
Aims: Age and the ε4 allele of apolipoprotein E (APOE) are the two greatest risk factors for Late Onset Alzheimer’s Disease (AD). APOE mediates its physiological effects in the brain by binding to cognate receptors like APOER2. Recent studies found a novel link between aging, neurodegeneration and alternative splicing. In AD patients, there is a subset of splicing changes unique to neurodegeneration in addition to age-associated splicing changes. APOER2 is highly alternatively spliced in neurons, and, in AD, there is less inclusion of APOER2 exon 18. APOER2 exon 18 is critical for ligand-induced long-term potentiation. We therefore hypothesize that APOER2 isoform distribution may be perturbed in AD contributing to synaptic and neuronal dysfunction.

Methods: To examine full-length APOER2 isoform distribution across the AD brain, we utilized long-read single molecule RNA sequencing to generate novel APOER2 transcript maps from the hippocampus and the parietal cortex of three female control individuals and three female Braak Stage IV AD patients.

Results: Across the human hippocampus and parietal cortex, we identified over 200 unique APOER2 transcripts that demonstrate combinatorial splicing events that may alter protein function. Several isoforms identified were unique to AD and demonstrate unique functional biology in in vitro assays compared to control-specific isoforms.

Conclusions: Our data indicates the combination of alternative splicing events in APOER2 is altered in AD and is critical to defining APOER2 receptor biology. We conclude that abnormal alternative splicing of receptors like APOER2 in aging and neurodegeneration may alter the interactome of key AD risk factor APOE.
COMBINING SINGLE CELL CHROMATIN LANDSCAPE AND GENE EXPRESSION WITH SPATIAL TRANSCRIPTOMICS TO CHARACTERIZE ALZHEIMER’S DISEASE PROGRESSION IN CRND8 APP MICE

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Aims: Alzheimer’s Disease (AD) currently impacts 6 million Americans who are living with the disease and 1 in 3 seniors die with a diagnosis of AD or dementia. Given the epidemiological state and impact of AD, a better understanding of the biology of the disease is needed to develop effective preventative and curative therapies. Here, we demonstrate the benefits of combining both single cell and spatial information in TgCRND8 APP transgenic mice to further understand amyloid deposition and its inflammatory signature.

Methods: Using the Chromium Single Cell Multiome ATAC + Gene Expression and Visium Spatial Gene Expression with FFPE assays, the open chromatin landscape and gene expression profiles of Tg APP-overexpressing and wild-type mice brains from 2 to 20 months old mice were evaluated. In addition, Aβ production and Aβ-associated neuroinflammation across several anatomical regions of the brain were analyzed and correlated with regulatory programs identified based on multiomic data.

Results: showed multi-cellular gene co-expression networks at both the single nucleus and spatial levels as well as distinct open chromatin sites during the course of amyloid deposition. These findings were noteworthy in brain regions containing Aβ plaques accompanied by a neuro-immune response from distinct cell populations as a function of amyloid deposition, e.g. C4b in oligodendrocytes within fiber tracts.

Conclusions: Together, the data showcases the utility of combining open chromatin and gene expression analysis at the single cell level with spatial transcriptomics of FFPE tissues to fully
characterize cellular networks and responses in the time-sensitive context of AD.
Aims: Histone acetylation mediated synaptic gene activation is critical for maintaining neural function, yet the neuroepigenetic mechanisms involved remain to be elucidated. We previously identified an imbalance in Tip60 histone acetyltransferase (HAT) and histone deacetylase 2 (HDAC2) in both the human Alzheimer’s disease (AD) hippocampus and the Drosophila AD brain that contributes to inappropriate repression of synaptic genes and cognitive decline. Further, we have shown that Tip60 regulates activity-dependent synaptic gene activation by shuttling into the nucleus of rat hippocampal neurons in response to extracellular cues. Here, we aim to determine whether nucleocytoplasmic shuttling of Tip60 and HDAC2 is disrupted in the AD brain using different yet synergistic AD Drosophila models that overexpress either APP or Aβ42.

Methods: Immunohistochemistry and confocal microscopy were used to visualize Tip60 and HDAC2 subcellular localization in the in vivo Drosophila brain.

Results: A robust decrease in Tip60 protein was observed in the nucleus and the cytoplasm in the AD adult Drosophila brain, both before and after plaque formation. In contrast, a significant increase in nuclear but not cytoplasmic HDAC2 protein was observed in late stages of AD brain neurodegeneration.

Conclusions: The alterations in Tip60/HDAC2 subcellular localization we observe in both early and late stages of AD in the Drosophila brain underscore the importance of appropriate chromatin regulator intracellular dynamics in cognitive centers of the brain. Future work will disrupt Tip60/HDAC2 shuttling mechanisms to explore the functional relevance of this process in activity dependent neural gene control and cognition in neural function and AD.
Aims: Biomarkers are an increasingly important field of research in the field of Alzheimer Disease (AD). The aim of this paper is to differentiate between AD and control patients using DNA methylation data and the k-nearest algorithm.

Methods: Methylation data from brain tissue of 68 individuals, including 34 control cases, was analyzed. Each patient had approximately 461,272 CpGs DNA methylation data. The algorithm controlled for the age and gender of the individual. The algorithm used was the k-nearest neighbors which is based on different estimates of the distance between the data. The algorithm was trained with half of the data and tested with the other half. One important factor when using this type of approach is to optimize the number of neighbors used (k). This was done empirically.

Results: In can be seen in the figure below that after a certain value increasing the number of neighbors (k) does not increase the accuracy of the forecasts. Furthermore, the process seems to reach a plateau after which forecasting accuracy is compromised. The accuracy of the method is nevertheless reasonable with a 70.6% successful classification rate.

Conclusions: The k-nearest neighbor algorithm can be applied to the problem of distinguishing between control individuals and AD patients using as an input CpG DNA methylation data. This classification
technique is likely impacted by the high dimensionality of the data (with more than 400,000 variables per patient). Reducing the dimensionality of the data is likely to help improving the accuracy and this could be an interesting area of future research.
APPLICATIONS OF NEURAL NETWORKS IN ALZHEIMER DISEASE METHYLATION BIOMARKERS

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Aims: As more data becomes available it is increasingly important to have techniques to process it efficiently. An example is the increase in CpG DNA methylation data achievable by using for instance Illumina methylation machines. The objective of this paper is to differentiate, using as input methylation data, between control individuals and patients with Alzheimer Disease (AD).

Methods: 68 patients were analyzed, with control cases accounting for 34 of the individuals. In each individual case the DNA methylation data of 461,272 CpGs was analyzed. The age and the gender of the patients were also included as inputs. An artificial neural network (ANN) was used as the classification algorithm with 15% of the data used as a testing dataset (not used during the training process). Only one hidden layer was included in the ANN with a 30 artificial neurons. 10 times cross validation was required in order to support the robustness on the model.

Results: The out-of-sample accuracy of the model reached 86.8%. Controlling for the age and gender of the patients appeared to be important. When these factors were not included the accuracy of the model declined below 66.2%.

Conclusions: DNA methylation can be used as an input for Artificial Neural Networks to differentiate between control patients and individuals with AD. Controlling for the age of the patient appeared to be important for the accuracy of the models. An interesting line of future research is analyzing the impact of adding additional hidden layers in what it is frequently called deep learning.
INTERACTION BETWEEN AMYLOID BETA AND PLATELET COLLAGEN RECEPTOR GLYCOPROTEIN VI

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Aims: The progression of Alzheimer's disease (AD) is associated with cerebral amyloid angiopathy (CAA). CAA is characterized by the deposition of amyloid-β peptides (Aβ), mainly Aβ40, in the walls of cerebral vessels. These amyloid deposits can reduce cerebral blood flow, trigger inflammation and lead to cognitive decline. Platelets express high amounts of the amyloid precursor protein (APP) and display all enzymatic activities necessary to produce amyloid β (Aβ) peptides. GPVI (glycoprotein VI) is the major collagen receptor at the surface of platelets and plays a critical role in platelet activation and platelet mediated inflammation. In this study, we investigated the impact of Aβ40 on GPVI at the platelet surface.

Methods: Cell culture experiments, FACS analysis, ELISA

Results: Binding studies showed that Aβ40 binds to GPVI and induces activation of the GPVI signaling cascade. Moreover, stimulation of platelets with Aβ40 led to externalization of GPVI to the platelet surface. Further analysis showed the formation of platelet-neutrophil aggregates (PNAs) in whole blood after incubation with Aβ40. Compared to wild type platelets, GPVI deficient platelets showed reduced production of reactive oxygen species (ROS) upon Aβ40 stimulation. Moreover, GPVI inhibition by antibody treatment as well as GPVI deficiency decreased platelet-mediated amyloid-β fibril formation in vitro.

Conclusions: Taken together, binding of Aβ40 to GPVI leads to activation of platelets and induces amyloid fibril formation.
Aims: Objectives Priming of the adaptive immune system by Alzheimer's Disease (AD)-associated brain-derived antigens is still a matter of debate. The existence of protective antibodies against β-amyloid aggregates in healthy aged donors indicates that the peripheral immune system can ‘sense’ the brain, most likely via drainage of brain-derived solutes through dural lymphatics. In exploratory immunophenotyping studies, we reported changes in T-cell profiles in blood of subjects with early cerebral β-amyloidosis. We hypothesize that β-amyloid-derived epitopes elicit specific adaptive immune responses detectable in the periphery starting in preclinical AD.

Methods: We designed cross-sectional and longitudinal immunophenotyping studies consisting of β-amyloid-PET-characterized blood donors, including healthy controls and mild cognitive impairment (MCI) patients. We analysed PBMCs via multiparameter mass cytometry and characterized clonally expanded cells via single-cell RNA-sequencing. Finally, we investigated T-cell specificity in vitro with antigen-presentation assays.

Results: We observed that increases in antigen-experienced subpopulations such as CD8+ TEMRA/effector T-cells in blood were associated with increased cerebral β-amyloid load and detectable as early as in preclinical AD stages. Moreover, we found memory and TEMRA/effector T-cells reactive against β-amyloid-derived epitopes in the blood of still β-amyloid-free cognitively healthy donors.

Conclusions: While the relationship between T-cell reactivity and cognitive outcomes in AD has been widely explored, the association of peripheral immune cell alterations with key pathological biomarkers such as β-amyloid deposition has not yet been studied in detail. Our study suggests that changes in adaptive immune responses might be detected already during early cerebral β-amyloidosis in a stage when cognitive deficits are not yet present.
THE NEURONAL ADAPTOR FE65 POTENTIATES NEURITE OUTGROWTH BY THE RECRUITMENT OF ARF6 AND ELMO1

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Aims: APP-binding family B member 1 (APBB1) is initially found to interact with APP to influence APP processing. Emerging evidence suggests that APBB1 participates in other neuronal processes including neurite outgrowth. As neurite atrophy/damage is often observed in many neurodegenerative diseases including Alzheimer’s disease and Parkinson’s disease, understanding the regulatory mechanisms of neurite outgrowth is essential for developing strategies to stimulate neurite regeneration. To this end, we have attempted to understand how APBB1 potentiates neurite extension by identifying novel APBB1 interactors.

Methods: Novel APBB1 interactors were identified by biochemical assays. The effects of the APBB1 interactors on neurite outgrowth and Rac1 activation were analysed by an enhanced Green Fluorescence Protein-based assay and Rac1 activation assay, respectively. Plasma membrane trafficking of APBB1 and the interactors were determined by biochemical fractionation and confocal microscopy.

Results: We have shown that the neuronal adaptor APBB1 activates neurite outgrowth by recruiting the small GTPase ARF6 and the Rac1 GEF ELMO1 by its PTB1 domain and N-terminal region, respectively. Interfering with the formation of the multimeric signalling complex attenuates both ARF6-ELMO1-mediated Rac1 activation and neurite elongation. The plasma membrane trafficking of ELMO1 is markedly decreased in cells with suppressed expression of either APBB1 or ARF6. Moreover, APBB1 is shown to increase the amount of ELMO1 in the recycling endosome, an organelle responsible for returning proteins to the plasma membrane.

Conclusions: In conclusion, APBB1 potentiates ARF6-Rac1 signalling by orchestrating ARF6 and ELMO1 to promote the plasma membrane trafficking of ELMO1 via the endosomal recycling pathway, and thus, potentiates Rac1-mediated neurite outgrowth.
VISUOMOTOR ASSOCIATIVE LEARNING IN PRODROMAL ALZHEIMER’S DISEASE

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Aims: A hallmark of Alzheimer’s disease (AD) is progressive memory impairment, with patients in the prodromal stage showing initial deficits in associative learning. Although numerous preclinical studies have used rodent models to examine how hippocampal dysfunction contributes to AD-related impairments in learning and memory, it remains unclear if early-onset deficits in striatal-dependent associative learning can help predict future cognitive decline.

Methods: Thus, to investigate the interplay between striatal-dependent associative learning and hippocampal dysfunction in prodromal AD, we are conducting cognitive-behavioural testing on 14-month old, male and female, transgenic Fischer 344 (TgAPP) rats that overexpress pathogenic human amyloid precursor protein, but do not spontaneously develop β-amyloid plaques. Hippocampal function is being assessed by the Morris water maze, whereas striatal-dependent associative learning is being tested with an operant-conditioning based visuomotor task that spans 18 days.

Results: Our preliminary results have revealed that, in the later days of the visuomotor task, TgAPP rats perform at lower accuracy than their wildtype counterparts; findings indicative of impaired associative learning. Furthermore, throughout the duration of testing, the female TgAPP rats are making their associative choices with slower reaction times, suggestive of greater task difficulty. Our ongoing experiments and analyses will determine if these striatal deficits precede or co-exist with hippocampal-dependent impairments in spatial learning and memory.

Conclusions: Ultimately, we anticipate that our preclinical data will provide novel insight into the interplay between striatal and hippocampal dysfunction in prodromal AD, as well as provide us with a rodent model to further investigate the underlying neuropathology.
Aims: Alzheimer’s disease (AD) and stroke frequently coexist but the mechanisms by which they interact are not well understood. Microglia play a critical role in mediating inflammatory responses in both stroke and AD. However, an accumulation of pro-inflammatory activated microglia can be detrimental and is associated with executive dysfunction. Minocycline is a tetracycline derivative that has been shown to effectively inhibit microglia activation. Using minocycline, we aim to assess the cognitive and neurohistological effects of microglia inhibition in a co-morbid model of AD and stroke.

Methods: A clinically relevant model of prodromal AD is the TgAPP21 rat which overexpresses a mutated form of the human APP gene but does not spontaneously develop plaques. Stroke was induced in 8–10-month-old male wildtype Fischer 344 and TgAPP21 rats by injection of endothelin-1 into the right dorsal striatum. Minocycline was administered for 4 days post-stroke, prior to testing for behavioural flexibility, learning and memory using an operant conditioning-based set-shifting task and the Morris water maze. Brains were histologically examined at 28 days post-stroke.

Results: Minocycline successfully reduced white matter microglia activation in both wildtype and TgAPP21 rats but did not affect astrocyte reactivity. Contrary to our hypothesis, minocycline did not have a significant effect on cognitive performance. Ongoing analyses will provide further insight into the effects of minocycline on brain pathology, as well as genotypic differences.

Conclusions: A critical association between microglia activation and cognitive decline has been established and this study further explores this association in the context of a co-morbid model of AD and stroke.
MOLECULAR AND FUNCTIONAL INTERACTION BETWEEN N-METHYL-D-ASPARTATE AND CANNABINOID CB2 RECEPTORS. CB2R-NMDAR HETEROMER UPREGULATION IN TRANSGENIC APP (SW,IND) MICE MODEL.

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Methods: Immunocytochemistry was used to analyze the colocalization between CB₂ and NMDA receptors; bioluminescence resonance energy transfer was used to detect CB₂-NMDA complexes. Calcium and cAMP determination, MAPK activation, and label-free assays were performed to characterize signaling in homologous and heterologous systems. Proximity ligation assays were used to quantify CB₂-NMDA heteromer expression in mouse primary cultures and in APP₅₆,₇₉ mice, an Alzheimer’s disease model.

Results: In a heterologous system we identified CB₂-NMDA complexes with a particular heteromer print consisting of impairment by cannabinoids of NMDA receptor function. The print was detected in activated primary microglia treated with lipopolysaccharide and interferon-γ. CB₂R activation blunted NMDA receptor-mediated signaling in primary hippocampal neurons from APP₅₆,₇₉ mice. Furthermore, imaging studies showed that primary cells (microglia or neurons) from APP₅₆,₇₉ mice displayed a marked overexpression of macromolecular CB₂-NMDA receptor complexes thus becoming a tool to modulate excessive glutamate input using cannabinoids.

Conclusions: The results indicate a negative cross-talk in CB₂-NMDA complexes signaling. The expression of the CB₂-NMDA Hets increases in both microglia and neurons from the APP₅₆,₇₉ transgenic mice, compared with levels in WT mice.
Aims: Deposits of Aβ in brain parenchyma and cerebral vessels are the pathological hallmarks of Alzheimer’s disease (AD). Recently, we demonstrated that aged Alzheimer transgenic mice (APP23), which develop amyloid-β deposits in the brain parenchyma and cerebral vessels, have pre-activated platelets in blood compared to aged control mice. Moreover, platelets adhere to vascular amyloid-β deposits and cause vessel occlusion in aged APP23 mice. A comprehensive analysis of these mice revealed that activated platelets directly contribute to vascular amyloid plaques. To investigate initial changes of platelets in these mice we analyzed platelets from middle-aged APP23 mice, which already have amyloid pathology in the brain parenchyma, but still no vascular amyloid deposits.

Methods: FACS and platelet function analysis

Results: Middle-aged APP23 mice had unaltered platelet count, platelet size and glycoprotein expression comparable to control mice. However, TEM analysis showed a significantly increased number of dense granules. Secretion of α- and dense-granules was increased selectively upon stimulation with CRP that stimulates the major platelet collagen receptor glycoprotein VI. Additionally, we observed enhanced CRP-triggered aggregation of platelets from middle-aged APP23. Flow chamber experiments revealed increased thrombus formation on collagen at high shear rates ex vivo but unaltered formation of thrombi under moderate shear. However, the release of vWF from platelet granules as well as binding to its receptor GPIb was unaltered in APP23 transgenic platelets.

Conclusions: APP23 mice show morphological and functional alterations of platelets before vascular amyloid plaques develop in these mice indicating that platelet changes in aged APP23 mice are not only the result of vascular Aβ deposits.
Aims: Alzheimer's disease (AD) is neuropathologically defined by the accumulation of amyloid β plaques in the brain parenchyma and vasculature. Mouse models of AD are important in preclinical drug discovery. To assess drug effects on the amyloid pathology of these models, reliable plaque detection and quantification methods are required. Here, we developed a light sheet fluorescence microscopy (LSFM) pipeline coupled with deep-learning image analysis, enabling unbiased, automated whole-brain 3D mapping and quantification of congophilic amyloid plaques in a mouse AD model.

Methods: Brains from 12-month-old double APP-PS1 transgenic (ARTE10, n=5) and age-matched wild-type (C57BL/6, n=7) mice were stained with Congo red. Brains were subsequently cleared and scanned on a LSFM. A deep-learning image analysis algorithm was trained and validated for whole-brain parenchyma and vasculature segmentation using the autofluorescence channel. Fluorescent congophilic deposits were then annotated, quantified, and anatomically mapped using a custom mouse brain atlas.

Results: Congophilic plaque quantification revealed significant increase in 93 out of 298 annotated brain regions. Among the top-20 regulated regions were subregions in the cortex (prelimbic area, anterior cingulate area, secondary motor area), hippocampus (subiculum, field CA1) and lateral septum. The vascular and parenchymal plaque segregation further unveiled detailed plaque deposition profiles of ARTE10 mouse brains.

Conclusions: We set a framework for using high-throughput, quantitative whole-brain 3D imaging in preclinical drug discovery for AD and other CNS diseases characterized by disseminating amyloid plaque pathology. In addition, the LSFM-deep learning platform allows for 3D visualizing whole-brain distribution of CNS drugs, including therapeutic antibodies targeting amyloid plaques.
INDUCIBLE GLOBAL AND NEURON-SPECIFIC MIR-34A OVEREXPRESSION AS NOVEL MURINE MODELS OF ALZHEIMER’S DISEASE PATHOGENESIS

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Aims: Alzheimer’s disease (AD), a chronic neurodegenerative condition resulting from pathological brain aging, is the most common cause of geriatric dementia, and is likely polygenic in origin. The discovery of microRNAs, small, endogenous, non-coding, and highly conserved RNAs that regulate post-transcriptional gene expression of potentially hundreds of genes, has revealed new drivers, and therefore therapeutic targets, of AD pathogenesis. Research from our group and others indicates that miR-34a may be a promising candidate.

Methods: To explore the role of miR-34a in vivo, we generated a global doxycycline (Doxy)-inducible miR-34a expression mouse model (miR34a\textsuperscript{+/-} 2X (TetR-TetO-miR34a). Three-month-old male and female miR-34a\textsuperscript{+/-} mice were treated for \~90 days with drinking water or Doxy (2mg/ml) to induce miR-34a expression and evaluated for domains disrupted in other transgenic AD mice. To ascertain brain- and cell type-specific vulnerabilities of miR-34a overexpression, we also generated a mutant mouse line in which miR-34a overexpressed is restricted to excitatory neurons of the central nervous system using a CaMKII\textalpha driver.

Results: Doxy-treated miR-34a\textsuperscript{+/-} mice showed T/Y-maze memory deficits, coinciding with upregulated miR-34a in all brain regions assayed. MiR-34a\textsuperscript{+/-} mice also showed evidence of known AD neuropathologies, including reduced glutamatergic receptor expression, altered APP processing leading to intracellular Aβ staining, and phosphorylated Tau. Experiments to determine consequences of brain-specific miR-34a overexpression are ongoing.

Conclusions: MiR-34a appears to contribute to an AD-like phenotype. Future work will leverage the utility of this Tet-inducible system to explore age-related susceptibility to, and capacity for recovery from, the cognitive and neuropathological consequences of miR-34a overexpression.
Aims: Our recent studies have addressed the correlation between sleep-wake rhythm fragmentation and risk of AD. We have demonstrated that experimentally-induced sleep fragmentation (SF) worsens AD-like neuropathology in female 3xTgAD mice. Due to sex differences in the risk of AD, this study investigated potential sex differences in the effects of SF.

Methods: We used an APPSwed/PS1(P264L) double knock-in (KI) model of AD-related pathology, and wild type (WT) controls, of both sexes (N=94 total). For three weeks, mice were exposed to SF or undisturbed sleep (US). SF consisted of 4 daily sessions (1 hour each) of enforced wakefulness, evenly interspersed throughout the light phase. During SF, mice were kept awake with novel objects and gentle stimulation with a paintbrush. Mice were individually housed in PiezoSleep cages (Signal Solutions LLC) for sleep recordings during weeks 1 and 3.

Results: SF caused a shift in percent sleep from the light phase to the dark phase (from ~2.3 : 1 to ~1.4 : 1; p<0.0001). This net shift was significantly larger in females than males (p=0.018). This difference was driven almost entirely by the female KI mice (p=0.005).

Conclusions: The much larger impact of SF on female KI mice as compared to either female WT or male mice suggests the existence of a sex-dependent interaction between sleep fragmentation, AD-related mutations, and pathology. Given the increased incidence of AD in women, understanding the mechanisms underlying this interaction may be of critical importance to advancing our understanding and treatment of this disease. Funded by NIH (AG068215).
TEMPORAL CHARACTERIZATION OF THE AMYLOIDOGENIC APP;PS1/APOE4 MOUSE MODEL OF ALZHEIMER’S DISEASE

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Aims: In clinical studies administering certain anti-beta-amyloid antibodies, vascular inflammatory adverse events have been reported. These events are observed as amyloid-related imaging abnormalities (ARIA). The frequency of ARIA events is higher among apolipoprotein (APOE) E4 allele carriers compared to non-carriers. With the intent of improving preclinical modeling of patients with Alzheimer’s disease who are most at risk for vascular complications of anti-beta-amyloid immunotherapy, we selected a recently developed mouse model: APP/PS1dE9 mice crossbred with human APOE E4 targeted replacement mice. The model has yet to be characterized at multiple ages. To evaluate face validity of the mouse model, we are conducting a battery of histological and biochemical tests.

Methods: To examine age-related pathological changes, we euthanized male and female APP/E4 mice across 3 ages: 8-, 12- and 16-months. Immunohistochemical methods and immunofluorescent staining are used to evaluate beta-amyloid plaque deposition, cerebral amyloid angiopathy, blood-brain barrier leakiness, neuritic dystrophy, and microhemorrhages. Additionally, we implemented immunoassays to quantify levels of beta-amyloid and APOE.

Results: ELISA of brain insoluble homogenates revealed significantly higher beta-amyloid40 and beta-amyloid42 levels in 16-month mice compared to 8- and 12-month mice. Furthermore, 16-month females showed significantly higher beta-amyloid40 levels than age-matched males. A significantly lower beta-amyloid (42/40) ratio was exhibited in 16-month mice compared to 8- and 12-month mice. Further analyses are underway.

Conclusions: Sex- and age-dependent increases in beta-amyloid deposition were observed in the APP/E4 mouse model. Upcoming analyses will better define the APP/E4 as a model for future preclinical anti-amyloid immunotherapy studies.
Aims: Selecting the optimal mouse model for target validation and preclinical testing is a major challenge that has limited progression of basic research discoveries in Alzheimer’s disease (AD) through the drug development pipeline. The inability to identify suitable model systems has numerous causes, such as differences in phenotypic measures used across models, mice that do not fully replicate disease pathophysiology, and restrictions on model use. To address these issues, the Model Organization Development and Evaluation for Late-onset Alzheimer’s Disease (MODEL-AD) consortium is generating new mouse strains that model late-onset, sporadic AD. These models are available without restriction to the research community.

Methods: MODEL-AD models are phenotyped with a standardized pipeline, and data is shared through the AD Knowledge Portal. We developed the MODEL-AD Explorer (https://modeladexplorer.org/) to allow easier exploration of phenotypic data.

Results: The Explorer shares gene expression and pathology data from 15 models. The pathology explorer quantifies immunostaining of microglia, astrocytes, dystrophic neurites, and amyloid plaques. The gene expression explorer can be used to compare differential expression of selected genes across models, sexes, and ages or to view all differentially expressed genes for selected mouse models as a function of age and sex. A third explorer shows the extent to which gene expression changes in mouse models mimic changes in humans with AD.

Conclusions: After evaluating phenotypic changes and selecting a model of interest, users can learn more about each strain by visiting the Jackson Laboratory website. The MODEL-AD Explorer will be continually updated to incorporate new phenotypic data from additional MODEL-AD strains.
THE EFFECT OF FASUDIL ON BRAIN AND BEHAVIOUR IN A RAT MODEL OF ALZHEIMER’S DISEASE

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Aims: Drug repurposing can help traditional drug development by speeding up the discovery of novel medicines for Alzheimer’s disease (AD) patients. The ROCK inhibitor fasudil is a strong candidate for repurposing for AD. Here, we investigate the effects of fasudil on AD-related pathology in the TgF344 rat model.

Methods: A total of 45 rats were used in this longitudinal investigation. 19 Wild-type (WT), 11 TgF344, and 12 TgF344 rats treated with fasudil underwent baseline MRI and MRS at 18 months of age. Then, fasudil was administered orally in drinking water (0.1 mg/ml) for 8 weeks. A set of behavioural tests (elevated plus maze (EPM) and frailty index) were performed during the last week of treatment followed by a second scan at 20 months of age. Data was then analysed with GraphPad Prism 9.2.

Results: Anxiety-like behaviour was shown to be significantly decreased in the fasudil-treated group compared to non-treated TgF344 animals (p<0.001). Furthermore, while MRS showed significant differences in N-acetylaspartate (NAA) levels in the transgenic group at both time-points, there was no discernible improvement after fasudil treatment. The analyses of additional structural and functional MRI are ongoing.

Conclusions: These findings suggest that fasudil is able to improve AD-related behaviour in animals even at a relatively advanced stage of pathology. Importantly, anxiety-like behaviour and overall frailty were ameliorated by 2 months of fasudil treatment. Further MR and biochemical analyses are planned and undergoing. These data support the case for repurposing of fasudil for treatment of AD in the clinic.
DEVELOPING AD MICROBIOME THERAPIES: AUTOMATED MEASUREMENT OF MOVEMENT DECLINE IN AB-EXPRESSING C. ELEGANS BY INHIBITING BACTERIAL FOLATE SYNTHESIS

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Aims: Fast and efficient testing in the early stages of drug development is required to develop new therapies for Alzheimer’s Disease. C. elegans models in which the spliced human Aβ peptide is expressed, show early paralysis and allow the study of the disease in a genetically malleable, short-lived whole organism. This animal model is maintained on live bacteria, providing a simplified model for microbe: host interactions.

Methods: Manual paralysis assays in C. elegans are subjective, time-consuming and slow. Here we present a methodology to provide high-quality and rich data that allows the development of new drugs that slow physiological decline. The Healthspan Machine provides non-invasive, continuous monitoring to study worm movement and speed in large populations.

Results: With this technology, we find previously unseen differences in patterns of decline across many models of neurodegeneration depending on the protein expressed and whether it is expressed in neurons or muscle. To assess the potential of this technology we applied drugs known to extend lifespan in C. elegans (rapamycin, metformin, etc.) to understand the intersection of neurodegeneration and its greatest predictor – age. In particular, the sulfonamide sulfamethoxazole (SMX) extends C. elegans lifespan by dampening folate synthesis in its laboratory food source of E. coli. Using a dose range, we find that SMX is more effective at preventing the decline of Aβ models than slowing ageing in wild type animals.

Conclusions: This result suggests that inhibiting folate synthesis in gut bacteria, or the downstream bacterial activities dependent on folate may be a new lead for treating neurodegenerative disease.
LOAD2: THE ROLE OF GENETIC AND ENVIRONMENTAL RISK FACTORS ON WHOLE BRAIN CONNECTOMICS

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Aims: Objectives: To understand whole brain connectomic changes in a novel MODEL-AD (Model Organism Development and Evaluation for Late-onset AD) mouse model that aligns with human LOAD in response to genetic and environmental risk factors

Methods: APOE4 and Trem2*R47H and humanized amyloid-beta (Aβ) allele were incorporated into C57BL/6J (B6) mice to produce LOAD2. LOAD2 mice of both sexes were fed either control or high fat diet (HFD), and were assessed via 18F-FDG PET/CT imaging for brain metabolism. Whole brain connectomic analysis was performed on atlas segmented brain regions, where hierarchical modularization was performed and statically compared across sex and treatment in mice at 12 months of age.

Results: Network analysis revealed three primary modules within network which were further partitioned into six secondary sub-modules of 3-7 brain regions/module. Brain regions within each sub-module contained either sensory (S) function, learning (L), or a mixture of motor, perception, and sensory (MPS) associated brain regions. Statistical analysis of the six sub-modules showed a significant sex and treatment dependent effect for LOAD2 mice on HFD by 12mo, which were module dependent.

Conclusions: The combination of APOE4, Trem2*R47H, and humanized Ab sequence with HFD induced age-dependent LOAD-relevant network changes consistent with brain circuit variations observed clinically, making this model and analysis a useful tool studying for disease mechanism and therapeutics testing.
A SPORADIC ALZHEIMER’S DISEASE MODEL IN MALE AND FEMALE RATS: BEHAVIORAL DIFFERENCES AND HIPPOCAMPAL IMPAIRMENT IN STREPTOZOTOCIN-INJECTED RATS

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Aims: To study the sporadic model of Alzheimer’s disease based on an intracerebroventricular injection of STZ (icv-STZ) using female rats, and to compare the effects with our previous results in males.

Methods: Sprague Dawley rats received a 3 mg/kg icv-STZ dose. Males were separated into Sham and STZ groups. Half of female rats were ovariecctomized (OVX) 14 days before icv-STZ injection and separated into Sham, STZ, OVX, and OVX+STZ groups. Two weeks post injection, we conducted the behavioral tests: Marble Burying (MB), Open Field, Novel Object recognition, Barnes Maze (BM), and Forced Swimming test (FST). Immunohistochemistry analysis for Iba1, GFAP and DCX were performed in hippocampus.

Results: STZ-males showed overt behavioral deficit, hippocampal damage, and neuroinflammation evidenced by reduced immature neurons and an increase in microgliosis and astrogliosis. In females, STZ affected behavioral performance differently depending on the presence of ovaries. Unlike males, STZ-females showed no differences in the MB, BM nor FST, versus control. Conversely, STZ altered all the behavioral parameters assessed in ovariecctomized rats. At the morpho-histochemical level, as well as males, STZ increased reactive microglia. Additionally, OVX+STZ group showed an increase in the total number of microglial cells. Unlike males, GFAP immunoreactive area was increased in the Dentate Gyrus (DG), in both STZ groups. Further, STZ reduced the number of immature neurons in the DG, but this loss was higher in OVX rats.

Conclusions: We confirmed the importance of conducting studies comparing sex differences. Besides, the ovarian status relevance in modulating icv-STZ neurodegenerative effects should be considered in future research.
NOVEL INHIBITORY NETWORK REMODELING IN KNOCK-IN MODELS OF ALZHEIMER’S DISEASE

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Aims: Aberrant neural networks are well established phenotypes in Alzheimer’s disease (AD). Subjects with AD suffer from higher rates of seizures, while in transgenic models such as the J20-hAPP line, animals display non-convulsive epileptic activity measured by EEG along with hyperexcitable parvalbumin (PV) expressing interneurons and ectopic sprouting of the inhibitory peptide neuropeptide-y (NPY) in the hippocampus. Although their widespread use as models of AD have allowed for a deeper insight into the disease’s pathological mechanisms, intrinsic limitations of transgenic models have led to the development of App knock-in animal lines. Here, we investigated the presence of altered inhibitory neural networks in hippocampal and subcortical regions of App knock-in lines.

Methods: To determine whether aberrant neural networks were present in 9-month-old C57BL/6J wild-type, AppNL (plaque-free) and AppNL-F (plaque-bearing) animals, sagittal sections were immunofluorescent stained for PV and NPY and cell numbers and neuronal fibers were quantified in hippocampus and striatum.

Results: Contrary to APP transgenic lines, we did not observe aberrant NPY hippocampal sprouting in either AppNL or AppNL-F mice. Surprisingly, AppNL-F mice displayed a decrease in NPY-positive fibers. This was not due to a population-specific loss of neurons, as both PV+ and NPY+ cell counts were unaltered across groups. In the striatum, NPY fiber density or cell counts were similar in both lines. However, PV+ neurons were lowered in the AppNL-F.

CIRCADIAN RHYTHMS OF TAU PHOSPHORYLATION AND SECRETION ARE DRIVEN BY BODY TEMPERATURE

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Aims: Alzheimer’s disease (AD) is characterized by the accumulation of beta-amyloid (Aβ) and hyperphosphorylated tau. Sleep disturbances are common in AD patients and worsen AD pathology. To unravel the relationship between sleep and AD, it is important to understand how circadian rhythms physiologically regulate Aβ and tau. Previous work has shown that tau secretion is driven by sleep-wake cycle but the pattern of tau phosphorylation during circadian rhythm is not known. Moreover, the impact of body temperature in tau phosphorylation and secretion are not known.

Methods: Tau was analyzed by Western blotting in the brains of awake and sleeping B6 mice (with permanent temperature recording). Tau secretion was assayed by ELISA and Dot-blotting in the medium of SH-S5Y5 cells overexpressing human Tau3R.

Results: We evidenced circadian variations in tau phosphorylation with tau being hyperphosphorylated during sleep. We demonstrated that temperature underlye these oscillations, as circadian variations in body temperatures are negatively correlated with tau phosphorylation. Preventing circadian changes in temperature abolishes tau hyperphosphorylation during sleep. We also observed that tau secretion was temperature-dependant, with higher temperatures increasing and lower ones decreasing tau in cell medium. At higher temperatures, tau-secreted species were less phosphorylated and more cleaved at D421 by caspase-3 and caspase-3 inhibition decreased tau secretion.

Conclusions: These data suggest that during sleep tau is more phosphorylated, less cleaved and less secreted than during wakefulness. These changes are driven mostly by body temperature. Since AD patients are prone to sleep disturbances and thermoregulation deficits, our study provides new directions for research and potential interventions.
HYPERPHOSPHORYLATION PROMOTES TAU SELF-ASSEMBLY INTO AMORPHOUS AGGREGATES ELICITING TLR4-DEPENDENT INFLAMMATORY RESPONSES

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**Aims:** Intracellular neurofibrillary tangles constituting hyperphosphorylated tau proteins are a pathological hallmark of several neurodegenerative diseases including Alzheimer’s disease. In particular, hyperphosphorylation has been suggested as a pathological switch, turning native tau into misfolded substrates and initiating aggregation. Hence, the aim of this study is to generate disease-relevant hyperphosphorylated tau and evaluate the structural and cytotoxicity profile of such aggregates.

**Methods:** Recombinant wild-type (WT) tau of isoform 0N4R was sequentially phosphorylated by PKA and SAPK4. LC-MS/MS and high-resolution native mass spectrometry were performed to determine the phosphorylation sites and the degree of phosphorylation. Super-resolution microscopy and electron microscopy were conducted to characterize the morphology of aggregates formed. The cytotoxicity of hyperphosphorylated tau aggregates in relation to the unmodified tau aggregates was evaluated using liposomal assay and human macrophage assay.

**Results:** As measured by LC-MS/MS and high-resolution native mass spectrometry, 0N4R tau was hyperphosphorylated at AD-specific epitopes in vitro. Fluorescence images along with TEM data demonstrated phospho-tau could self-polymerize into nonfibrillar, amorphous aggregates while WT tau formed short fibrillar aggregates. In comparison with the unmodified aggregates, which required heparin induction, these self-assembled hyperphosphorylated tau aggregates more efficiently disrupt membrane bilayers and induce Toll-like receptor 4 (TLR4)-dependent inflammatory responses, as parameterized by calcium transients, ROS production, and proinflammatory cytokine releases (TNF-α, IL-1β, and RANTES).

**Conclusions:** This study demonstrates that hyperphosphorylated tau can spontaneously polymerize into amorphous aggregates at physiological concentrations. These hyperphosphorylated tau aggregates were more cytotoxic than heparin-induced WT aggregates through diverse mechanisms, providing mechanistic insights into how tau hyperphosphorylation is potentially damaging to cells.
POSTERS

DISULFIDE BOND FORMATION IN MICROTUBULE-ASSOCIATED TAU PROMOTES ITS ACCUMULATION UNDER OXIDATIVE STRESS

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**Aims:** Accumulation of tau is thought to underlie neuron loss in a group of neurodegenerative disorders. Tau is an intrinsically disordered protein, and its structure is regulated by posttranslational modifications. Oxidative stress promotes the neurodegenerative processes, and antioxidant defense systems are known to protect against tau toxicity. Tau has two cysteine residues (C291 and C322) that can interact with other tau molecules or other proteins via thiol-disulfide exchange. Since cysteine residues can form disulfide bonds in response to oxidative stress, C291 and C322 may mediate the effects of oxidative stress. However, the roles of these cysteine residues in tau toxicity in vivo are not fully understood.

**Methods:** We established a series of transgenic flies carrying 2N4R tau with known mutations or deletions that alter aggregation propensity.

**Results:** Oxidative stress causes tau accumulation via disulfide bonds formation. We found that C291 and C322 in human 2N4R tau (tau^{wt}) form disulfide bonds. Analyses of the flies expressing tau with alanine substitutions at these sites (tau^{C291/322A}) revealed that these cysteines contribute to the stability of tau. Expression of tau^{wt} in the fly retina causes eye degeneration, while tau^{C291/322A} expression generated much less neurodegeneration than tau^{wt}. Next, we co-expressed superoxide dismutase 1 (SOD1) with tau^{wt} or tau^{C291/322A}. SOD1 reduced tau protein levels, while tau^{C291/322A} to a lesser extent than tau^{wt}, suggesting that cysteine residues contribute to tau accumulation caused by oxidative stress.

**Conclusions:** Oxidative stress causes tau accumulation via formation of disulfide bond in tau, resulting in enhanced neurodegeneration. Cysteine modifications may be an effective strategy to suppress tau accumulation and toxicity.
Aims: The presence of p-tau in biofluids has previously been proposed to be a response to neurofibrillary tangle pathology. However, the increase of p-tau in cerebrospinal fluid (CSF) precedes detectable neurofibrillary tangle pathology, as indexed by tau PET, by up to a decade, suggesting that soluble tau could be an indication of early tau pathology. With this study, we investigated the heterogeneity of p-tau species in CSF in order to assess the clinical status of participants of the TRIAD cohort.

Methods: Support vector machines were used to identify cutoff values of p-tau181, p-tau217, p-tau231 and p-tau235 in CSF, both individually and combined, to separate a group of AD patients (n=25) and young controls (n=28). Using these cutoff values, signatures were calculated on an individual level in a group of individuals with cognitive impairment (n=62) and age-matched controls (n=75). Additionally, [18F]MK6240 SUVR maps and memory composite scores were calculated and evaluated using the Logical Memory test, the Rey Auditory Verbal Learning Test, the Face Name Association Test and the Free and Cued Selective Reminding Test.

Results: When combining different CSF p-tau species, the largest contribution came from p-tau181, followed by p-tau217, p-tau235 and p-tau231. Achieving the cutoff for multiple p-tau species was associated with higher Braak stages (fig 1) and lower memory composite scores (fig 2). In particular, achieving the cutoff value for p-tau217 was associated with later Braak stages. Figure 1:
Conclusions: Our findings suggest that heterogeneity in p-tau species carries predictive power in the identification of incipient Alzheimer’s Disease.
RETINAL GANGLION CELL VULNERABILITY TO TOPOGRAPHICAL-SPECIFIC TAUOPATHY IN PRODOMAL AND CLINICAL ALZHEIMER’S DISEASE

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Aims: Key pathological hallmarks of Alzheimer’s Disease (AD) in the brain include aggregates of amyloid-β (Aβ) and hyperphosphorylated tau (pTau). There is growing evidence that the retina, a central nervous system (CNS) tissue, exhibits AD pathology like the brain. Retinal Aβ was identified in subjects with mild cognitive impairment (MCI) and AD, variably accumulating across cell layers and subregions. Further, loss of retinal ganglion cells (RGCs) was documented in these patients. While increased pTau was also reported, our understanding of retinal pathology associated with AD remains obscure. Moreover, the topographical distribution of retinal pTau in AD and cell-type susceptibility is understudied.

Methods: Immunohistochemistry was used to quantify retinal pS396Tau in different subregions and cell layers and in RGCs expressing ribonucleic acid binding protein with multiple splicing (RBPMS). Manual count and immunoreactive-area of RBPMS-positive RGCs and pTau-containing RGCs were assessed in postmortem retinae of subjects with normal cognition (NC), MCI, or AD.

Results: A significant 45% decrease in RBPMS-expressing RGCs was revealed in MCI and AD retinae. RBPMS cell loss was accompanied by a 2-3-fold pS396Tau increase in prodromal and clinical AD retinae, especially in mid-peripheral subregions. While pS396Tau is densely observed in the outer plexiform layer (OPL), colocalization of pTau inside RBPMS-RGCs is also pronounced. Elevated pS396Tau in remaining RBPMS-RGCs may drive their displacement to deeper retinal layers in disease states.

Conclusions: This study reveals vulnerability of RBPMS-expressing RGCs to pTau accumulation and degeneration. Future studies should investigate retinal cell-type susceptibility to tauopathy, including amacrine and horizontal cells in AD.
INHIBITION OF THE TAU AGGREGATES THROUGH OF MICHAEL ADDITION USING COMPOUNDS ISOLATED FROM THE ANTARCTIC

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Aims: Prevent the formation of protein aggregates in tau by covalent interactions between protein and 3 compounds obtained from Antarctic lichens.

Methods: Treatment of plant material, isolation and purification of compounds using chemical extraction techniques. Production of the Tau 4R fragment (htau244-372) recombinantly in microbial systems and its purification by chromatographic techniques. Fluorescence tests by thioflavin (ThT) to monitor the inhibition of Tau aggregation in the presence of compounds of interest. Observation of structures by atomic force microscopy. Labeling of maleimide and Total Internal Reflections Fluorescence Microscopy (TIRFM). Culture assays in N2a neuroblastoma cell lines, immunofluorescence and lactate dehydrogenase (LDH) test.

Results: Inhibition of the progression of aggregation in tau by the interaction of cysteine with the compounds of interest by covalent bonds formed by Michael addition.

![Chemical structures](image-url)
**Decrease of the content of β sheets in tau aggregates.** N2a cells incubated with oligomers in the presence of compound 2 (A) avoid changes in their morphology and decrease membrane damage in relation to cells treated with control oligomers (B).

**Conclusions:** Compounds 1, 2 and 3 to 50 μM inhibit the advance in the formation of aggregates in tau. The mechanism of Michael's addition is presented as an alternative to prevent the advance in the formation of cytotoxic tau oligomers by interaction of an unsaturated carbonyl group α, β and cysteine. Compound 2 remodels soluble oligomers and decreases leaf content β. Oligomers treated with compound 2 do not cause membrane damage in N2a cell culture compared to aggregates alone.
TDP-43 PROMOTES ENHANCED TAU NEUROTOXICITY AND SELECTIVE NEURODEGENERATION

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Aims: TDP-43-positive inclusions are present as a co-pathology in over half of patients with Alzheimer’s disease (AD). AD patients with TDP-43 pathology have a faster disease course, more rapid cognitive decline, and increased neurodegeneration, but the molecular mechanisms underlying this are unknown. It is possible that synergies between pathological proteins drives worsened outcomes in these patients.

Methods: We have developed new models of multi-pathology AD by combining expression of human TDP-43 with tau or amyloid beta (Aβ) pan-neuronally in C. elegans. To characterize these transgenic animals, we measured changes in behavior, pathological protein accumulation, and neuron loss over time.

Results: TDP-43 enhances tau but not Aβ neurotoxicity, resulting in exacerbated uncoordinated locomotion, neuronal dysfunction, accumulation of phosphorylated tau, and progressive neurodegeneration. We show suppression of tau neurotoxicity protects against tau and TDP-43 synergism. We further identify subsets of neurons that are selectively vulnerable to co-morbid tau and TDP-43.

Conclusions: Characterizing the relationships between pathological proteins in AD is critical to understanding processes underlying disease. Our new models of protein co-expression demonstrate a specific relationship between tau and TDP-43 may drive worsened outcomes in AD patients with co-morbid TDP-43.
TAU SEEDING IN THE HEALTHY BRAIN

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Aims: Accumulation of intracellular assemblies of the microtubule-associated protein tau (MAPT) underlies the group of diverse neurodegenerative diseases termed tauopathies. The origin of sporadic tauopathies remains elusive amidst a growing body of evidence that suggests tau can behave in a prion-like manner and that seed-competent tau monomer (M₃) itself can encode the information required for templated aggregation of tau assemblies. The transition from inert to seed-competent tau species is proposed to underlie the critical step to pathology. However, it remains possible that seed-competent tau strains exist that do not represent pathology.

Methods: We have developed an antibody (MD3.1) that efficiently isolates low amounts of tau seeds from healthy brain. Using highly specific next-generation ultra-sensitive tau biosensors combined with immunopurification with MD3.1, we detected significant seeding activity in an age-diverse cohort of tauopathy negative control brain.

Results: Seeding activity was detected in the parietal cortex, while the cerebellar cortex was absent of detectable tau seeds. We observed no correlation between seeding activity and age. Tau isolated from the cortex of aged human tau knock-in mice showed no seeding activity.

Conclusions: Our results suggest that tau seeds are present at low levels in the cortex of all healthy adult human brains and the presence of tau seeds in healthy brain has regional and species specificity. Given tau’s ability to efficiently propagate strains in vivo, and the presence of tau seeds in healthy adult human cortex, we propose that its ability to assemble into self-replicating structures may reflect a normal function that goes awry in disease states.
AD-BRAIN DERIVED TAU OLIGOMERS AND SARKOSYL-INSOLUBLE TAU FIBRILS HAVE SIMILAR SEEDING ACTIVITIES

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\textbf{Aims:} Alzheimer's disease (AD) progression has recently been associated with the propagation of fibrillar Tau species, but it remains unclear which tau species are responsible for propagation. Both soluble Tau oligomers and sarkosyl insoluble fibrils have been implicated in tau seeding. We now directly compare for the first time the seeding activity of soluble Tau oligomers and sarkosyl-insoluble Tau fibrils (SARK) derived from the same AD brains.

\textbf{Methods:} We extracted both oligomers and SARK Tau from the frontal cortices of 6 Braak VI AD and 2 control non-AD subjects. We compared the bioactivity of these Tau species in a FRET-based biosensor cell line.

\textbf{Results:} As expected, AD-derived Tau oligomers and SARK Tau have higher seeding activity than their counterparts derived from control non-AD patients. Oligomeric tau, prepared from a soluble fraction and isolated over size exclusion column, has a similar bioactivity to SARK tau from the same brain (Cohen's effect size d=1.0). Interestingly, if the SARK fibrils are sonicated, their seeding activity is enhanced (Cohen's effect size: d = 1.7). Tau specificity of the effect was verified by immunodepletion of Tau, which showed greatly reduced seeding activity.

\textbf{Conclusions:} Oligomeric and non-sonicated SARK Tau have similar seeding activities across AD brains; this is consistent with the hypothesis that both fractions contain analogous conformations to promote seeding. Sonication of SARK Tau leads to increased seeding activity as already reported in previous studies. Studies of their neuronal uptake and toxicity mechanisms could further reveal their respective mechanistic contribution to AD pathology.
Aims: Cell to cell transfer of pathogenic Tau is known to be a critical step in the process of disease propagation throughout the brain. Comprehensive understanding of the process in which neurons internalize Tau protein from the extracellular space is important for developing disease progression prevention strategies. However, our knowledge about the molecular and cellular mechanism of Tau uptake is still very limited.

Methods: Here we used a high throughput live assay to study the neuronal uptake and intracellular accumulation of heparin-induced Tau aggregates.

Results: Our results showed that neurons internalize and accumulate aggregated Tau at higher extend compared with monomeric Tau. Further analysis revealed that different genes play role in the uptake of monomeric and aggregated Tau.

Conclusions: In conclusion, our data indicate that Tau monomers and aggregates are efficiently taken up by neurons, while using differential uptake mediators. This suggest that it might be possible to inhibit prion-like spreading of pathogenic Tau without interfering with the physiological transmembrane flow of normal protein. These findings can pave the way for developing therapeutic strategies to hamper disease progression.
Aims: Evolution of unique tau strains favoring the most aggressive and toxic conformers is currently debated in the context of cell-to-cell transmission and neuronal vulnerability hypothesis of Alzheimer’s disease (AD). Primary neuronal cultures with increasing ratio of 4R to 3R tau isoforms through the maturation are the golden standard tool in neurobiology studies. The propagation and pathogenesis of aggregated tau conformers isolated from sporadic and rapid progressive AD cases can be investigated in mouse primary neurons.

Methods: Primary neurons at various times of evolution and tau isoform ratios are inoculated with structurally characterized human brain-derived tau strains isolated by PTA-precipitation from AD brain homogenates. The misfolding and aggregation of endogenous mouse tau is monitored by antibodies selective against pathological forms of tau and mouse tau. Cytotoxicity, western blots, confocal microscopy, and conformation-dependent immunoassays are applied to quantify the aggregation rate of endogenous mouse tau, and alterations of neuronal morphology.

Results: The range of tau aggregation rate is higher in cases of rapid progressive AD compared to sporadic AD and correlates with the amount of exogenous tau added. Significant morphological changes associate with dendritic projections. Interestingly, the degree of tau isoform ratios in primary neurons affect the propagation of tau misfolding and aggregation.

Conclusions: Our data show that rodent primary neurons are a valuable cell model to study mechanisms of tau strain replication. Although the species barrier likely affects the tau transmission, and misfolding propagation, the tau strains from various AD phenotypes can be still differentiated by their distinct neuronal impact.
A hallmark of Alzheimer’s Disease (AD) is the progressive accumulation of neuronal inclusions of insoluble Tau, which correlates with the severity of cognitive decline in AD patients. The spread of Tau pathology seems to occur via transneuronal, trans-synaptic propagation of aggregated tau along anatomically connected brain regions. This suggests that interfering with tau propagation could modify disease progression. Commercially available multichamber systems have been used to investigate Tau propagation across synaptically connected neuronal populations in vitro. However, their low-throughput characteristics make them unsuitable for quantitative testing of therapeutic interventions targeting tau propagation. 

Methods: Here, we developed a neuronal tau propagation assay using microfluidics plates in 384-well format containing 96 units with three chambers each, connected by microfluidic channels. hiPSC-derived tau-mutant neurons were cultured in all three chambers and population 1 (P1) neurons were seeded with exogenous Tau fibrils. The formation of Tau aggregates in the connected populations P2 and P3 was analyzed 10-weeks later by quantifying somatic tau aggregates immunolabeled with the MC1 antibody using high-content image analysis.

Results: Tau aggregation was found in all neuronal populations. Using tau-KO neurons for P1 or P2 significantly lowered aggregation levels in connected P2 and P3 neurons, respectively. We furthermore find seed concentration-dependent increase of tau propagation and show that siRNA-mediated tau knockdown in P2 and P3 neurons significantly attenuates tau propagation.

Conclusions: In summary, we developed a high-throughput tau propagation assay platform using functional neurons, which allows for siRNA-based gene silencing and could enable genetic and pharmacological screens of tau propagation modulators in vitro.
Aims: Transmission of seeding-competent Tau is postulated to propagate Tau pathology in Alzheimer’s disease (AD) brain. However, a biomarker assay for direct detection of Tau seeding in biofluids is still lacking. We therefore developed a Tau seed amplification assay (SAA, aka. RT-QuIC) for AD brain and cerebrospinal fluid (CSF) using full-length 0N3R Tau as substrate.

Methods: Using Tau SAA we investigated the relationship between the levels of different Tau species and their seeding activity in over 100 brain samples from three AD/control cohorts and in antemortem AD/control CSF.

Results: Analysis of AD brains revealed a correlation of Tau SAA signal with seeding activity in a cellular seeding assay, the levels of phosphorylated Tau-212/214 and oligomeric Tau, but not with total Tau. Seeding-competent Tau was detected in AD hippocampus, but not in matched AD cerebellum from the same individuals, demonstrating specificity of the assay. We identified increased seeding activity in brains with higher Braak stages (0-VI), suggesting that Tau SAA signal reflects the amount of pathological Tau inclusions in the brain. Our most recent data suggests that Tau SAA adapted for antemortem CSF accurately discriminates AD patients with high CSF pTau from controls with low CSF pTau.

Conclusions: SAA accurately reflects Tau pathology in the AD brain and provides an alternative for early detection and studies of seeding mechanisms. Excitingly, we detected seeding-competent Tau in antemortem AD CSF providing a basis for the investigation of seeding-competent Tau in AD patients.
Aims: Our main goal of the study is to determine if an enriched environment will have some effect on the amount and propagation of tau neurofibrillary pathology in the rat tauopathy animal model.

Methods: To reach the experimental goals we employed the SHR72 rat transgenic animal model, which expresses human truncated tau 151-391 aa and the presence of NFTs are predominantly in the brainstem, but no NFTs are present in the hippocampus area. To examine if an enriched environment has some effect on propagation and induction of tau pathology we induce tau pathology in the hippocampus by intracerebral applications with sarkosyl insoluble isolates from Human Alzheimer’s Disease patients. During the study, we monitor also effect on cognition with the Morris Water Maze test. Postmortem we analyze brain tissue on an immunohistochemical level with several phospho dependent antibodies.

Results: We observed that an enriched environment had a shrinkage effect on the number of tangles immunopositive for tau pathology and furthermore have also a positive effect on some cognition aspects compared to rat in standard housing conditions evaluated as a control group.

Conclusions: Our results suggest that an enriched environment can be used as a possible successful nonpharmacological approach for improving cognition and can slow down the propagation of tau pathology in AD and other tauopathies.
SEEDING AND SPREADING PROPERTIES OF TAU IN RAPIDLY PROGRESSIVE ALZHEIMER’S DISEASE

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Aims: Clinical phenotypic heterogeneity of Alzheimer’s disease (AD) is well established. The rapidly progressive subtype of AD (rpAD), often misdiagnosed as Creutzfeldt-Jakob disease, has a specific phenotype (unusual symptoms, shorter disease duration and faster cognitive decline than classic AD (cAD)) that may be related to a different tau strain.

Methods: We investigated the properties of tau aggregates from patient brain preparations through different approaches: biochemistry (sucrose density gradient ultracentrifugation) (n=5 cAD, n=5 rpAD, n=2 CTRL cases), in vitro (Tau RD P301S FRET Biosensor cells) (n=2 cAD, n=2 rpAD, n=2 CTRL) and in vivo (hippocampal inoculations in the P301S mice) (n=8 cAD, n=8 rpAD, n=8 CTRL) studies.

Results: Pathological tau from cAD patients showed a higher propensity to seed aggregation and spread than that from rpAD cases: 1) Tau pathology was seen at a greater distance in cAD than in rpAD-injected P301S mice, 2) Tau aggregates were more numerous in Tau RD P301S FRET Biosensor cells exposed to cAD vs rpAD homogenates, 3) Tau in cAD patients formed denser assemblies, as seen with sucrose density gradient ultracentrifugation.

Conclusions: Through different approaches we showed that pathological tau species in cAD and rpAD have distinct seeding and spreading properties suggesting the presence of different tau strains. The paradoxical results we obtained with rpAD brain extracts (low seeding and spreading potential) suggest that the specificity of the rpAD variant might rely on a predominance of specific small (eg oligomeric) tau assemblies that remain to be identified.
Aims: In Alzheimer’s disease (AD) and related tauopathies, somatodendritic missorting of the axonal Tau protein is a major pathological hallmark. Previous work suggests a critical role for the axon initial segment (AIS) in maintaining polarized Tau distribution. However, the exact mechanisms of AIS-mediated Tau trafficking, i.e. AIS-specific interaction partners and necessary Tau domains or motifs are unclear.

Methods: We perform sorting analysis using a Tau construct library, EB3-based live-cell imaging and leading-edge proximity labelling techniques in three different neuronal cell models (e.g. hiPSC-derived glutamatergic neurons) to elucidate the interplay between Tau and AIS components during the process of axonal Tau sorting.

Results: First results revealed the insufficiency of the Tau N-terminal half to target the axonal compartment. Further, successful Tau sorting in SH-SY5Y-derived neurons questions the previously reported importance of the AIS proteins ANKG and TRIM46 for i) axonal Tau enrichment, and ii) microtubule orientation at the AIS. To successfully implement the TurboID interaction assay in neuronal cultures, we started to introduce fusion constructs of Tau and the promiscuous biotin ligase BirA into hiPSC-derived neurons via lentiviral delivery. When established, this will help to unravel the Tau interactome domain- and sorting-dependent by using truncated or modified Tau in the first step, and to conduct compartment- and even AIS-specific Tau interaction studies in the future.

Conclusions: Gaining these data would not only improve the understanding of axonal Tau enrichment in healthy neurons, but it would help to understand the detrimental cascade underlying pathological Tau missorting in AD and other tauopathies.
A DROSOPHLA MODEL DEPICTING BRAAK-LIKE PROPAGATION OF TAU PATHOLOGY

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**Aims:** Prion-like propagation through circuits is believed to be the mechanism by which tau pathology spreads throughout the brain in tauopathies like Alzheimer’s disease (AD). This is reflected in the neuropathological Braak-staging of disease and manifests in the progressive cognitive decline evident clinically.

**Methods:** Though various synaptic proteins are implicated, the precise players and mechanism(s) mediating the trans-cellular spread of pathological tau species remain unclear. Furthermore, though the trans-cellular spread of pathological tau species has been demonstrated in many experimental models, the neurobiological consequences in recipient neurons are largely unknown. Moreover, in all such studies, the tau species that propagates is invariably mutated or isolated from pathological fractions of brains of tauopathy patients. This is puzzling given that it is wild-type tau that becomes pathological and spreads in AD, and this process is accompanied by neurodegeneration.

**Results:** We report a novel Drosophila model in which wildtype human tau expressed in select neuronal subsets becomes pathological and undergoes trans-cellular spread through adult brain circuits, causing neurodegeneration and behavioural impairments reminiscent of late stages of disease in AD brain. The superior genetic tractability of this model makes it ideally suited for dissection of the key players that mediate this pathogenic process through genetic and pharmacological modifier screens.

**Conclusions:** Furthermore, the availability of functional and behavioural assays for many adult brain circuits will enable future studies to more directly reveal the neurobiological consequences of spreading tau pathology.
Aims: Periodontitis, a chronic systemic inflammatory disease, is a common problem in the elderly. With increasing recognition that inflammation plays a key role in the pathophysiology of Alzheimer’s disease (AD), we aimed to define the roles of cytokines in the pathogenesis of periodontitis and AD.

Methods: WT and 3xTg AD mice were injected with heat-killed P. gingivalis into their buccal mucosa three times per week every other week for a total of 5 weeks. Sickness Behavior and cognitive functions were assessed through open field, spontaneous Y maze and puzzle box test. Different brain regions were harvested for further analysis.

Results: WT and AD mice injected with heat-killed bacteria had increased periodontal bone loss, which was accompanied by increased gene expression levels of IL-1b and TNF-a in the gums. Behavioral tests revealed that bacterial injection worsened long-term memory functions in WT mice and exacerbated both short- and long-term memory functions in AD mice. Immunofluorescent staining revealed increased intensity levels of phospho-tau as well as microglia in the brains of both WT and AD mice injected with heat-killed bacteria.

Conclusions: Our study revealed that increased cytokine immune responses in the gums was accompanied with brain inflammation and increased tau pathology in WT mice and exacerbated pathological features in AD mice. The increased levels of IL-1 beta and TNF-alpha observed from the gums of both WT and AD mice indicates their potential role in the pathogenesis of both periodontitis and AD.
INTRAVENOUS INJECTION OF PHF-TAU PROTEINS FROM ALZHEIMER BRAIN EXACERBATES NEUROINFLAMMATION, AMYLOID BETA AND TAU PATHOLOGIES IN APP/PS1 TRANSGENIC MICE

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Aims: Alzheimer’s disease (AD) is characterized by the accumulation in the brain of intraneuronal aggregates of abnormally and hyperphosphorylated tau proteins and of extracellular deposits of amyloid-β surrounded by dystrophic neurites. Experimental models have shown that tau pathology propagates in the brain after intracerebral or intraperitoneal injection of brain homogenates or pathological tau (PHF-tau) from AD brains. Further investigations are however necessary to assess the potential role of extracerebral routes on tau pathology spreading in the brain, e.g., through the intravascular route.

Methods: In this study, we have analysed the effect of intravenous injection of PHF-tau from AD brains on the formation of tau and amyloid pathologies in the brain of wild-type and of the amyloid 5XFAD mice model.

Results: We observed that 5XFAD mice with a disrupted blood brain barrier showed increased plaque-associated astrogliosis, microgliosis, and increased deposits of Aβ40 and Aβ42 after injection of AD PHF-tau. In addition, an increased phosphotau immunoreactivity was observed in plaque-associated dystrophic neurites.

Conclusions: These results suggest that the medical use of blood products containing PHF-tau proteins should be carefully considered in view of a potential exacerbation of neuroinflammation and AD pathologies.
POSTERS

TOXOPLASMA GONDII INFECTION EXACERBATES TAUOPATHY IN AN ALZHEIMER’S DISEASE MOUSE MODEL.

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\textbf{Aims:} Toxoplasma gondii (Tg) is one of the most common zoonotic pathogens, infecting a wide range of animals as well as \textasciitilde 1/3 of the human population. Infection of immuno-competent individuals is characterized by parasite-containing cyst development in the brain, leading to life-long latent infection. So far, the interplay between latent cerebral toxoplasmosis and AD remains largely unknown. The present project aimed at determining the link between chronic toxoplasmosis and tauopathy.

\textbf{Methods:} We evaluated the impact of chronic toxoplasmosis, triggered early on later tauopathy development in the THY-Tau22 mouse model of tauopathy. 4w-old Tau22 mice and control littermates were infected with type II parasites, causing most infections in Humans, and mice were sacrificed 6 months post-infection, a stage Tau22 mice exhibit limited neuroinflammation. We then performed biochemical and molecular investigations to determine the hippocampal (HPC) impact of infection.

\textbf{Results:} We detected Tg cysts in the HPC of infected mice. We observed a significant increase of Tau hyperphosphorylation/aggregation and inflammatory markers in the HPC of Tau22 infected mice vs. non-infected mice. Bulk RNA-Seq analysis of HPC indicated that the magnitude inflammatory changes promoted by cerebral toxoplasmosis in WT animals was particularly enhanced under the Tau22 background. Finally, we found that Tg infection correlates with activation of the kynurenine pathway in the HPC of Tau22 mice, suggesting a detrimental impact of quinolinic acid.

\textbf{Conclusions:} Together, our results suggest that cerebral toxoplasmosis might prime the brain to the later development of Tau-pathology and neuroinflammation, possibly contributing to AD-like pathogenesis. This link is now under scrutiny in AD patients.
TDP-43 PATHOLOGY IN ALZHEIMER’S DISEASE SIGNIFICANTLY INFLUENCES MICROGLOIOSIS AND ASTROGLOIOSIS

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Aims: Microgliosis and astrogliosis play a critical role in Alzheimer’s disease (AD) pathogenesis, but their precise contributions to the disease process are currently unknown. Here we evaluated differences in microgliosis and astrogliosis in the hippocampus among three neuropathologically-defined AD subtypes: hippocampal sparing (HpSp) AD, typical AD, and limbic predominant AD.

Methods: In order to determine the contribution of AD pathologies to neuroinflammation in AD, 20 cases of each subtype (n=60 total) were neuropathologically evaluated on serial sections of the hippocampal in the CA1 subsector with CD68 (activated microglia), GFAP (astrocytes), tau, amyloid-β (Aβ), and Transactive response DNA-binding protein 43 kDa (TDP-43) immunohistochemistry. Digital pathology was employed to quantify immunohistochemical staining.

Results: The levels of both microgliosis and astrogliosis were lowest in HpSp AD, whereas typical AD and limbic predominant AD have similar levels suggesting a ceiling effect, or plateau, of gliosis levels. There was not an appreciable difference in early tau versus advanced tau markers in hippocampal CA1 among AD subtypes. Multivariable regression modelling showed a significant relationship between CD68 immunoreactivity (representing microgliosis) and tau and TDP-43 pathology levels, and GFAP immunoreactivity (representing astrogliosis) with Aβ, tau, TDP-43, and APOE ε4 genotype. By far the highest predicted increase of glial burden was associated with phosphorylated TDP-43 burden, which predicted an approximately 1.5-fold increase in CD68 and GFAP burden.

Conclusions: These findings highlight the significant role TDP-43 pathology plays in affecting the microenvironment and interplay between neuroinflammation and neurodegeneration, and have important implications for therapeutic strategies in AD.
CHARACTERIZATION OF DISTINCT LRRK2 VARIANTS LINKED TO PARKINSON’S AND INFLAMMATORY BOWEL DISEASE

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Aims: Chronic inflammation and the gut-brain axis are suggested to play a critical role in the pathogenesis of Parkinson’s disease (PD). Mutations in the LRRK2 gene represent the largest known cause of heritable PD, occurring in up to 40% of select patient populations. Intriguingly, the recent identification of LRRK2 variants associated with both PD and Crohn’s disease, a subtype of inflammatory bowel disease (IBD), has provided genetic basis to link these two disorders. This raises the question of whether certain type of PD and IBD share the same disease origin and mechanism of progression. The aims of this project are as follows: 1. Characterize how the N2081D risk variant increases risk of Parkinson’s and Inflammatory bowel disease. 2. Investigate the differences and commonalities with PD-causing LRRK2 G2019S variant.

Methods: We have developed a knock-in mouse model of the LRRK2-N2081D Crohn’s-Parkinson’s disease risk variant. In order to validate this model, we have employed an inducible colitis model. In our experiments, dextran sulphate salt (DSS) is added to the drinking water, causing progressive destruction of epithelial tissues leading to inflammation and weight loss that characterizes inflammatory bowel disease.

Results: Our findings demonstrate that N2081D mutation increases substrate phosphorylation in a small subset of previously defined LRRK2 targets. We have also observed that mice harboring the LRRK2-N2081D mutation exhibit a dramatically increased sensitivity to induced colitis as determined by weight loss, histological analysis, spleen weight, colon length and survival.

Conclusions: Herein, we present a novel, pathogenically validated LRRK2 knock-in disease model.
Aims: Brains of AD patients are characterized by an early synaptic loss. We wondered if and how the AD susceptibility gene BIN1 impacts synapses. Considering that BIN1 possesses over 10 isoforms, we aimed to assess isoform-specific effects of BIN1 on synaptic structure and connectivity.

Methods: We generated Drosophila transgenic lines expressing brain, muscular and ubiquitous human BIN1 isoforms 1 (BIN1iso1), 8 (BIN1iso8) and 9 (BIN1iso9), respectively. We performed electroretinograms on BIN1 isoform-expressing retina followed by immunofluorescence and electron microscopy. We further analyzed BIN1 synaptic toxicity in rat primary hippocampal neurons using tricompartiment microfluidic devices. BIN1iso1 or BIN1iso9 were overexpressed in the pre- or postsynaptic compartments using lentiviruses and synaptic connectivity was analyzed by distance-based assignment of postsynaptic puncta to presynaptic puncta.

Results: In Drosophila, we observed that BIN1iso1 expression induced a loss of electrophysiological activity of photoreceptor neurons and involved the loss of the ON and OFF transients, reflecting the loss of synaptic activity. This was specific to BIN1iso1, suggesting that synapses are sensitive to BIN1iso1-induced toxicity. In addition, we observed abnormal, gigantic vesicles at the presynaptic terminals. In rat neurons, we further compared BIN1iso1 and BIN1iso9 by overexpressing them selectively in the pre- or post-synaptic neurons. We observed a loss of synaptic connectivity only when expressing BIN1iso1 in the presynaptic compartment.

Conclusions: Our results suggest that BIN1 has an isoform-specific, deleterious effect on synaptic integrity when expressed in the presynaptic terminal. This effect could contribute to the synaptic loss observed early in AD.
HYPEREXCITABILITY INDUCED BY WILD-TYPE HUMAN TAU IN PRIMARY NEURONAL CULTURES

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Aims: Alzheimer’s disease (AD) is characterized by the pathological accumulation of amyloid-β (Aβ) and Tau in the form of Aβ plaques and neurofibrillary tangles (NFT), respectively. At the synaptic level, early Aβ pathology leads to neuronal hyperactivity. However, the role of Tau in neuronal excitability is still controversial. Some studies have observed that, in mice, co-expression of human Aβ and mutant Tau leads to the suppression of neuronal activity, overcoming the hyperactivity induced by Aβ. In contrast, other studies have suggested an increased excitability associated with the expression of human Tau. Thus, in the present study we aim to elucidate the role of Tau in neuronal excitability in the presence and absence of elevated Aβ.

Methods: Primary neuronal cultures from WT and APP/PS1 transgenic mice were transduced with human Tau (WT and P301L), and the changes in neuronal excitability were evaluated by measuring fluctuations in calcium levels. Furthermore, neuronal cultures were assessed by immunocytochemistry.

Results: After 7 days of expression of human Tau, we could observe that the expression of WT Tau led to an increase in calcium spikes, but not amplitudes, in WT and APP/PS1 primary neuronal cultures. In contrast, the expression of Tau P301L did not clearly alter the neuronal activity in the cultures.

Conclusions: Our results suggest a differential role in the effect of human Tau on neuronal excitability, indicating an altered effect associated with the overexpression of WT human Tau.
Aims: Anatomical studies show earliest signs of tau pathology in stellate-cell islands in entorhinal cortex (ERC) layer II. However, the molecular mechanisms that confer vulnerability to ERC layer II cells early in AD is unknown. Our research showed early calcium dysregulation in layer II ERC, where phosphorylated tau accumulated on the calcium-storing smooth endoplasmic reticulum (SER) under glutamatergic synapses, and PKA-phosphorylated ryanodine receptors on SER showed evidence of calcium leak. cAMP-PKA magnification of calcium is observed in PFC, associated with HCN channel opening to dynamically regulate synaptic strength. This process is regulated by phosphodiesterases (PDE), regulation that is lost with age. We examined whether this “signature of flexibility” could also be seen in layer II ERC, underlying vulnerability to tau pathology with aging.

Methods: We used high-spatial resolution immunoEM to localize PDE4D and HCN1 in young rhesus macaque (8-10y) ERC layer II.

Results: PDE4D and HCN1 were primarily observed in postsynaptic compartments in macaque ERC layer II. In dendritic spines, PDE4D was concentrated on SER spine-apparatus and in postsynaptic density, and HCN1 expressed in membranes near excitatory synapses. Within dendritic shafts, PDE4D labeling was observed along microtubules and near mitochondria, whereas HCN1 was organized along the plasma membrane.

Conclusions: PDE4D is optimally positioned to modulate cAMP microdomains and control calcium extrusion from SER. HCN1 channels are localized to facilitate dynamic physiological representation of sensory experience governed by cAMP-PKA signaling. Anatomical patterns in ERC layer II corroborate data in vulnerable glutamatergic circuits in PFC, suggesting conserved molecular properties in association cortices most susceptible in AD.
OVEREXPRESSION OF AN FTLD MUTANT OF TAU DISRUPTS THE TAU INTERACTOME AND ENHANCES NETWORK EXCITABILITY IN PRIMARY CORTICAL NEURONS

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Aims: Frontotemporal lobar degeneration (FTLD) can be caused by autosomal dominant mutations in the MAPT gene, such as P301L. In vitro, P301L tau is hyperphosphorylated and mislocalizes to the somatodendritic region and dendritic spines, where it can affect synaptic function. The aim of this project was to investigate how this mislocalisation influences the profile of tau binding partners and alters synaptic signalling and network function.

Methods: Primary mouse cortical neurons were transduced with adeno-associated viruses containing eGFP-tagged human tau constructs including the P301L tau mutant under the control of the neuronal specific hSyn1 promoter. Tau phosphorylation was analysed by immunoblotting. The tau interactome was identified through immunoprecipitation with a GFP-trap followed by isobaric labelling and tandem mass spectrometry. This enabled quantitative comparison between the WT and P301L tau interactomes. A microelectrode array was used to assess effects on network activity.

Results: Phosphorylation at the Ser262 phospho-epitope was increased with P301L tau compared to WT tau. Analysis of the tau interactome highlighted differential binding to proteins involved in transcription, proteasomal degradation and synaptic calcium signalling. Micro-electrode array analyses demonstrated enhanced baseline excitability in neurons transduced with P301L tau compared with WT tau or GFP controls.

Conclusions: We have successfully generated a neuronal model of tau overexpression that forms active networks within two weeks. Using this model, we have demonstrated that P301L mutant tau has the potential to affect key pathways that regulate cellular and synaptic function and increase network excitability.
INTRACELLULAR AGGREGATION OF TAU OR ALPHA-SYNUCLEIN IS A PRE-REQUISITE FOR THE FORMATION OF GRANULOVACUOLAR DEGENERATION BODIES

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Aims: Granulovacuolar degeneration bodies (GVBs) are an early hallmark of tauopathies that has also been reported in association to misfolded alpha-synuclein. However, the attribution of GVBs to a protein aggregate is challenging in post mortem tissue, where co-occurrence of different proteinopathies is commonly found. In this study, we make use of in vitro models and post mortem tissue to assess whether intracellular aggregation of tau is required for the formation of GVBs.

Methods: Patient-derived tissue as well as primary mouse neurons to model tau or alpha-synuclein pathology are employed. Aggregation of tau and alpha-synuclein is achieved in vitro by seed-dependent and seed-independent methods. Data are acquired by using confocal microscopy and analyzed by ImageJ.

Results: Our data show increased levels of pathological tau in neurons containing GVBs in Alzheimer’s Disease patients, also when no obvious pathology is present. We show for the first time that alpha-synuclein aggregation also triggers the formation of GVBs in vitro in the absence of tau pathology. Lastly, we demonstrate that GVB formation in vitro is not seed-dependent by employing spontaneously aggregating models.

Conclusions: We conclude that intracellular aggregation of tau or alpha-synuclein is required for GVB formation in the human brain as well as in vitro.
INVESTIGATING THE CONTRIBUTION OF AN INTRONIC VARIATION AT THE TRIM11/TRIM17 LOCUS TO PATHOLOGICAL AND CLINICAL HETEROGENEITY IN PROGRESSIVE SUPRANUCLEAR PALSY

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Aims: Progressive supranuclear palsy (PSP) is a primary tauopathy characterized by pathological accumulation of the protein tau, and presents as up to 9 distinct clinical phenotypes. To enhance therapeutic strategies that prevent and delay aberrant tau aggregation, an improved understanding of tau clearing mechanisms and genetic modifiers of phenotype and/or progression is required. Recently, an intronic variant in the proteasome-related TRIM11 gene (or TRIM17 in linkage disequilibrium) was identified as a potential modifier of PSP phenotype. The objective of the current work is to investigate if TRIM11/TRIM17 are involved in altering the accumulation of pathological tau.

Methods: To determine if tau is a substrate for the enzymatic activities of TRIM11/TRIM17, their interaction with tau was investigated by co-immunoprecipitation (co-IP) in unseeded or seeded HEK cell biosensors. Resultant interactions were further characterized by immunocytochemistry (ICC) and immunohistochemistry (IHC) in FTD patient fibroblasts directly-converted to neurons, and in a novel mutant human tau knock-in mouse model of tauopathy.

Results: Co-IP and ICC/IHC identified that tau interacts with TRIM11 and TRIM17 only when tau is aggregated with patient brain material. Interestingly, while TRIM11 is either partly co-localized with, or adjacent to tau inclusions, TRIM17 directly localizes with tau inclusions. This interaction dichotomy is highly suggestive that TRIM11 and TRIM17 have distinct roles when in complex with tau inclusions.

Conclusions: These findings provide molecular evidence that the ubiquitin proteasome system is involved in the processing of pathological tau. Future work will focus on elucidating if TRIM11 and/or TRIM17 can mitigate the formation or clearance of pathological tau aggregates.
Aims: Previous studies of our group showed that the abnormal tau protein impairs mitochondrial function and dynamics. Mitochondria are coupled to the endoplasmic reticulum (ER) via mitochondria-associated ER membranes (MAMs), which are known to be altered in Alzheimer’s disease (AD). An important MAMs function is the regulation of cholesterol synthesis and transfer to mitochondria. Therefore, we aimed to elucidate the impact of disease-associated tau on the MAMs with a specific focus on cholesterol homeostasis.

Methods: We used SH-SY5Y cells expressing mutant tau (P301L) and the corresponding vector-expressing cells to study the impact of Tau on the mitochondrial morphology, the ER-mitochondria contacts, and intracellular cholesterol homeostasis.

Results: In the presence of abnormal tau, mitochondria showed an elongated shape, together with impairments in the ER-mitochondria contacts. Free cholesterol level was increased in total cell lysates but were decreased in mitochondria isolated from the P301L-tau expressing cells compared to the control (Vector) cells. We also detected an abnormal cholesterol distribution within the cells, as well as disturbances in the expression of genes related to cholesterol homeostasis when mutant tau was expressed.

Conclusions: Abnormal tau expression alters the intracellular cholesterol metabolism and transfer to mitochondria in a process that might involve dysfunctional MAMs. The study of underlying mechanisms is still ongoing, and key experiments are currently performed in vivo (pR5 mice). These findings may highlight specific targets for therapeutic intervention. This work was supported by grants from the Synapsis Foundation - Alzheimer Research Switzerland ARS, and the University of Basel Research Fund.
Aims: Aging is a risk factor for several of the world’s most prevalent diseases, including Alzheimer’s disease (AD). Since mouse models have some limitations regarding transferability to human physiology and lifespan, suitable human in-vitro models to investigate age-related bioenergetics defects are wanted. Aging is triggered by exposure to acute and chronic stress during life. Therefore, the aim of this study is to characterize and validate in-vitro models of aging, especially “stress”-induced aging.

Methods: We used to characterize the bioenergetic profile of young vs aged donors in human fibroblasts (HFs). Bioenergetic parameters include total ATP level, mitochondrial membrane potential (MMP), mitochondrial respiration, glycolysis, and the mitochondrial morphology. Then, we accessed whether different stress approaches mimic the effects of aging on bioenergetics. Among the stressors, we tested the effect of the human glucocorticoid cortisol (main stress hormone) and the mitochondrial stressor rotenone on young HFs.

Results: A decline of ATP, MMP and mitochondrial respiration and a rise of glycolysis was observed in aged HFs compared to young HFs. The mitochondrial morphology was characterized by a more fragmented mitochondrial network in aged HFs. For the "stress"-induced aging condition, cortisol-induced a decrease in ATP and MMP, but no effects were observed on the other bioenergetics. Rotenone presented the same effects than aging, except for the mitochondrial morphology.

Conclusions: Based on our data, the chosen stressors seem only to mimic aging partially on some bioenergetics parameters. Further investigation must be done to understand aging in vitro and consequently the underlying mechanism leading to AD, such as tau pathology.
ACCUMULATION OF NEURONAL DEBRIS BY ASTROCYTES - A POSSIBLE MECHANISM FOR SPREADING OF TAU PATHOLOGY

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Aims: In the Alzheimer disease (AD) brain, tau inclusions are frequently found in astrocytes. However, the mechanism behind the appearance of these deposits and their relevance for disease progression is unknown. We have previously shown that astrocytes, in addition to aggregated proteins, engulf dead cells. In this project, we are investigating astrocytes ability to promote cell-to-cell spreading of toxic tau aggregates following ingestion of dead neurons with tau pathology.

Methods: To induce robust tau pathology in cultured hiPSC-derived neurons, we exposed the neurons to synthetic tau seeds (oligomers and fibrils) for up to 2 weeks. Immunocytochemistry (ICC) was used to assess toxic effects of the seeds and western blot analysis (WB) was performed to evaluate changes in tau-phospho variants. To induce apoptosis, the neurons were exposed to an UV-burst and the dying neurons were then co-cultured with hiPSC-derived astrocytes. ICC was used to measure the astrocytes' degradation capability.

Results: Neurons treated with tau-oligomers displayed a stark changed morphology. In addition, oligomer, but not fibril treatment lead to a significant reduction in the number of synaptophysin positive puncta. However, WB indicated an increase in several phospho-variants as well as the general kinase GSK3-b in neurons exposed to tau-fibrils, but not to oligomers. Importantly, astrocytes were shown to ingest and accumulate the neuronal cell corpses.

Conclusions: Tau-oligomers are more toxic than fibrils, but fibrils possess greater seeding capacity. Astrocytes are poorly equipped to handle neuronal corpses and may be involved in the spreading of pathological tau.
TAU PATHOLOGY AND ASTROGLIAL REACTIVITY: A COMPARATIVE STUDY OF TWO MOUSE MODELS OF TAUOPATHY

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Aims: Astrocytes are becoming crucial players in the context of neurodegenerative proteinopathies, such as Alzheimer’s disease (AD). Astrogial response has been mainly analyzed in amyloidogenic scenarios, but less is known about their involvement in tauopathies. Here, we aimed to analyze astrogial reactivity to hyperphosphorylated-tau (ptau) in the hippocampus of two transgenic mouse models of tauopathy, ThyTau22 and P301S (2- to 12/18-months).

Methods: Proteinopathy was assessed by western-blotting and immunohistochemistry (AT8). Neuroinflammation was analyzed by qPCR and bright-field immunohistochemistry, glial-ptau relationship by confocal and transmission electron microscopy.

Results: P301S mice exhibited an intense reactive astrogliosis, increasing progressively with aging accordingly to a strong ptau accumulation, whereas ThyTau22 model showed slighter astrogliosis related to lesser proteinopathy. P301S astrogliosis correlated with an acute DAM-like microglial activation, not observed in ThyTau22 hippocampus. In both models, reactive astrocytes contained ptau, especially around vessels.

Conclusions: Our results support that astrocytes respond to ptau in the absence of Abeta. This reactivity correlates with tau pathology and depends on microglial DAM-like activation. In addition, reactive astrocytes may play a role in the elimination/spreading of ptau species through the brain. Deciphering the mechanisms underlying these processes might allow the development of strategies to slow down the progression of AD and other tauopathies.
Aims: The possible production of dopaminergic neurons from human umbilical cord-derived mesenchymal stem cells (HUC-MSCs) is a major breakthrough for neural tissue engineering and clinical treatment of neurodegenerative diseases like Parkinson’s disease. The main goal of the current study is to determine the differentiation potential of HUC-MSCs into dopaminergic neuron-like cells.

Methods: HUC-MSCs were isolated and cultured on Matrigel and induced with a cocktail of dopaminergic neuronal differentiation factors. The capacity of HUC-MSCs for differentiation into dopaminergic neuron-like cells was assessed by real-time PCR, immunocytochemistry and high-performance liquid chromatography (HPLC), and then compared with the differentiated cells in the cell culture plate.

Results: The differentiation assessment at the level of mRNA and protein illustrated that Matrigel could significantly increase dopaminergic neurons markers compared to the culture plate.

Conclusions: Overall, the results suggest that HUC-MSCs can successfully differentiate dopaminergic neuron-like cells on Matrigel, having great potentials for the treatment of dopaminergic neuron-related diseases.
Aims: In order to better understand the links between tau and glucose homeostasis, the present study aimed at investigating the metabolic phenotype of a new knock-in (KI) mice model.

Methods: Males and females tau KI mice model expressing a human tau protein bearing the P301L mutation under the control of the endogenous mouse Mapt promoter and their non-transgenic littermates (referred as WT) were used. A complete metabolic phenotyping was explored under high fat diet (HFD) versus CHOW diet in both sexes. Also, glucose-stimulated insulin secretion (GSIS) was studied using isolated islets from tau KI and tau knock-out mice and mouse β pancreatic cell line (MIN6).

Results: While under chow diet tau KI mice do not exhibit significant metabolic impairments, we could observe that under HFD male, but not female tau KI animals exhibited glucose homeostasis alterations as compared to control littermates. Interestingly, using immunofluorescence, tau protein was found colocalized with insulin in the b cells of pancreatic islets. Additional GSIS experiments performed on isolated islets from tau KI and tau knock-out mice revealed that both exhibit impaired insulin secretion, an effect recapitulated in the mouse β pancreatic cell line (MIN6) following tau knock-down.

Conclusions: Altogether, our data suggest that loss of tau function in pancreatic β cell might favor the development of glucose homeostasis impairment and could contribute to metabolic changes observed in AD.
THE EFFECT OF METABOLIC DISTURBANCES ON MOUSE BRAIN PATHOLOGY DEPENDING ON ALZHEIMER’S DISEASES RISK

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Aims: The incidence of Alzheimer's disease (AD) increases in metabolic syndrome. APOEɛ4 allele is the strongest risk gene in AD and it also enhances metabolic syndrome like obesity and insulin resistance. However, their interaction in inducing AD pathology is controversial.

Methods: Human APOEɛ3 (3KI) and APOEɛ4 knock-in (4KI) mice were used to examine apoE isoform-specific effects. And APOE knockout mice (KO) were included to see the effect of apoE itself. These mice were fed on normal chow (ND) or high-fat chow (HFD) for 24 weeks from 12 week-old. And the brain pathology was examined.

Results: The increase in fasting blood glucose levels with HFD was most remarkable in 4KI mice, which was higher than in KO. The insulin levels in cortical extracts were highest in KO mice with HFD than in 3KI and 4KI mice. The levels of phospho-IRS1 at Ser616, a marker of insulin resistance, were not different between ND and HFD in 4KI mice. It was quite different from those of 3KI and KO mice: pIRS-1 (Ser616) levels increased in 3KI but decreased in KO mice after intake of HFD. To see the effect of metabolic changes on brain pathology in terms of neurodegeneration and inflammation western blot was performed. The presynaptic protein expression level was lower in 4KI and KO mice than in 3KI, but this difference disappeared with HFD.

Conclusions: These findings suggest that 3KI mice are susceptible to HFD-induced insulin resistance and synaptic degeneration compared with 4KI mice. The underlying mechanism will be examined further.
Aims: Alzheimer’s disease (AD) is a complex disorder and multiple cellular and molecular mechanisms are involved in AD onset and progression. Recent evidences has suggested that metabolic alterations are an important pathological feature in disease progression in AD. Likewise, diabetes and obesity, two major metabolic illnesses, are risk factors for AD. These two overwhelming diseases are associated with a significant expansion of white adipose tissue. Here, we hypothesize that the white adipose tissue may serve as a key communicator organ between the brain and peripheral metabolic illnesses and affecting both types of disorders.

Methods: We used histological stains, immunohistochemistry and biochemical means to determine changes in the white adipose tissue from WT and db/db mice. Moreover, similar techniques were used in the brain of 3xTg-AD mice that received white fat pads from WT and db/db donors to determine any changes in amyloid and tau pathology.

Results: Our study shows that recipient 3xTg-AD mice from db/db fat pads develop profound changes in tau pathology due to increased CDK5 expression. Moreover, adipose tissue transplanted from donor WT and db/db mice into recipient 3xTg-AD mice indicate that db/db associated white fat tissue induced profound tau pathology changes in recipient 3xTg-AD mice.

Conclusions: Overall, our study demonstrate a novel important crosstalk between Alzheimer’s disease and diabetes type II through white adipose cells. A more profound understanding in these processes may turn in novel and promising therapeutic strategies for AD and metabolic illnesses.
Dysregulated Locus Coeruleus Firing Coincides with Anxiety-Like Behaviors in Young TGF344-AD Rats

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Aims: Hyperphosphorylated tau pathology, a hallmark of Alzheimer’s disease (AD), appears first in the noradrenergic locus coeruleus (LC), and emergence of prodromal symptoms consistent with altered LC activity parallels accumulation of hyperphosphorylated tau in the LC. In rodents, the LC engages compensatory mechanisms in response to damage to maintain normal function, such as lesion-induced increased firing rate of surviving neurons. How tau alters LC firing rates, whether AD-like pathology triggers compensatory mechanisms, and if these changes influence behavior are unknown.

Methods: 6- and 15-month TgF344-AD rats, which overexpress AD-causing human APP and PS1 mutations and develop early endogenous tau pathology in the LC, and wild-type (WT) littermates were used for these experiments. Baseline and evoked activity of LC units were recorded. A separate group of age-matched rats was tested on the elevated plus maze (EPM), open field (OF), and novelty suppressed feeding (NSF) to assess anxiety-like phenotypes.

Results: LC neurons from TgF344-AD rats exhibited lower basal firing rates compared to WT littermates. Significantly higher mid-phase responses following 5ms 10mA footshocks were noted in 6-month TgF344-AD rats compared to age matched WT littermates. Trends towards increased footpinch-induced activity, elevated signal-to-noise ratio, and shorter interspike interval were also apparent in 6-month TgF344-AD rats, but were not statistically significant. No differences in anxiety-like phenotypes in the EPM or OF were observed, but TgF344-AD rats at both ages took longer to eat during NSF.

Conclusions: These results suggest potential compensatory mechanisms to maintain normal LC function in the presence of AD-like neuropathology that may have deleterious behavioral consequences.
Aims: Tau is involved in maintaining neuronal structure. In tauopathies, tau can aggregate to form oligomers (oTau). Although the toxicity of oTau is well established, the mechanistic basis of its actions on neuronal function remains poorly understood. Previously, full-length recombinant oTau was found to disrupt neuronal function, synaptic transmission and plasticity (Hill et al, 2019). In this study, we look to understand how oTau mediates these changes.

Methods: We truncated the tau molecule into two parts: the first 123 amino acids and the remaining 124-441 amino acids. We have used these clinically relevant truncations to elucidate the mechanisms underlying the changes in neuronal properties. We introduced the truncated versions of tau in aggregated form into single hippocampal pyramidal cells in acute mouse brain slices and measured the resultant changes in neuronal properties.

Results: These truncated tau molecules had specific effects on neuronal function, allowing us to assign the actions of full-length tau to different regions of the molecule. We identified one key target for the effects of tau, the voltage-gated sodium channel, which could account for the effects of tau on action potential waveform.

Conclusions: This simple, yet highly effective technique of introducing structurally defined aggregated proteins into single neurons allows unparalleled levels of detail and provides a unique opportunity to understand the underlying pathology for tauopathies. By truncating the tau molecule, we have probed the mechanisms that underlie tau dysfunction, and this increased understanding of tau’s pathological actions will build towards developing future tau-targeting therapies. Hill et al (2019).eNeuro,6(5),pp.eNEURO.0166-19.2019.
Aims: Several Tau-lowering strategies are being evaluated as a potential treatment for AD. However, the mechanisms that regulate Tau – encoded by the MAPT gene – expression are not fully understood. MAPT antisense 1 (MAPT-AS1) was recently identified as a long non-coding IncRNA (lncRNA) associated to MAPT and proposed to repress MAPT translation. We independently explored the potential roles of MAPT-AS1 on Tau expression in neurons.

Methods: We evaluated MAPT-AS1 and MAPT expression and spatial distribution in human iPSC derived models and in human brain samples from control and AD individuals. We performed gain- or loss-of-function experiments using antisense oligonucleotides (ASOs), short interference RNAs (siRNAs) and lentiviral overexpression constructs to modulate MAPT-AS1 expression in human neuronal models in vitro.

Results: We used stranded RNA-seq and CAGE-seq to determine MAPT-AS1 identity in human CNS. We found that MAPT-AS1 is co-expressed with MAPT in human brain samples. Additionally, MAPT-AS1 localizes predominantly in the cytoplasm of human neurons in situ and its expression increases with neuronal maturation. We utilized custom-made ASOs, siRNAs and lentiviral overexpression constructs that dose-dependently alter MAPT-AS1 levels. However, increasing or reducing MAPT-AS1 levels had no effect on MAPT transcription, splicing or Tau translation in our models.

Conclusions: We demonstrated that neuronal MAPT-AS1 does not regulate general MAPT expression at the transcriptional or post-transcriptional level. We encourage the scientific community to continue exploring other mechanisms that regulate Tau expression to support the Tau-targeting strategies currently in clinical trials.
Aims: In AD pathology, the accumulation of tau in neurofibrillary tangles correlates closely with neuronal death in affected brain areas and cognitive decline. Nevertheless, NFT formation can also be observed sporadically in cognitively normal elderly individuals, independent of neuronal loss, which suggests either the presence of additional pathomechanisms, or the loss of protective mechanisms that act synergistically with tau aggregation specifically in AD patients. The aim of this study is to identify such mechanisms in unbiased screening approaches using iPSC-derived cortical neurons.

Methods: We developed a genome wide CRISPR-mediated KO screen in hiPSC-derived cortical neurons. We generated a MAPT-mutant hiPSC line with inducible expression of Cas9 and an integrated genome wide sgRNA library. Differentiated KO neurons were treated with Tau seeds to induce endogenous tau aggregation. We hypothesize that cells, in which protective mechanisms are disrupted by genetic KOs, are vulnerable to increased tau aggregation and therefore are lost during the screen. Remaining cells were collected and sequenced for the prevalence of sgRNAs.

Results: Upon induction, Cas9-hiPSCs robustly expressed Cas9 that induced genetic knock outs in an efficient manner. KO iPSCs were successfully differentiated into neurons and treated with Tau seeds. Remaining cells were collected and subjected to next generation sequencing.

Conclusions: Bioinformatic analysis will identify candidate protective genes and pathways associated with cellular vulnerability upon KO in the presence of tau aggregation-inducing conditions. Selected genes will be validated for their impact on tau aggregation as well as neuronal health in various in-vitro and in-vivo models and consolidated with existing AD databases.
ACIDIC NANOPARTICLES RESTORE AUTOPHAGY FUNCTION BY ENHANCING LYSOSOMAL ACIDIFICATION AND RESCUE TAU-INDUCED CELL DEATH

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Aims: Alzheimer’s disease (AD) is a neurodegenerative disorder characterized by the presence of tau inclusions in affected brain regions. While the large insoluble neurofibrillary tangles have been the histopathological hallmark of AD, the soluble tau oligomers that are formed prior to fibril formation has been recently proposed to be the principal toxic species. Both cellular and mouse models of tauopathy and AD have indicated that endolysosomal autophagy dysfunctions contribute to tau accumulation and play a role in AD pathogenesis. Most studies have focused on promoting specific lysosomal enzyme activity as a viable strategy to restore lysosome function. In this study, we aim to achieve a direct lowering of lysosomal pH to enhance its luminal acidification in order to increase all enzyme activities and promote clearance of toxic tau aggregates.

Methods: We synthesized a novel type of acidic nanoparticles (acNPs) that is capable of lowering pH to induce lysosome acidification. We applied the acNPs to SH-SY5Y and N2a cells overexpressing tauP301L and characterized autophagy function, extent of tau accumulation and spreading as well as tau-induced cell death.

Results: We showed that restoration of lysosomal acidification activates autophagy function, reduces tau accumulation and rescues tauP301L induced cell death. Treatment of acNPs also reduced tau secretion into the cell culture media, suggesting a potential role in preventing tau spreading.

Conclusions: We propose acNPs as a tool to study the mechanism of lysosomal acidification as well as a potential therapeutic for AD and other neurodegenerative diseases in general.
THE CHAPERONE ACTIVITY OF S100B PREVENTS TAU AGGREGATION AND SEEDING

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Aims: Tau is implicated in the formation of oligomers and fibrillar aggregates that evade proteostasis and spread between cells. Tau pathology is accompanied by neuroinflammation, and some early inflammatory mediators encompass protective functions. This is the case of S100B, an astrocytic Ca²⁺-binding protein augmented in AD that inhibits Aβ aggregation [1,2]. Here we aimed to determine its broader role in proteostasis regulation by investigating interactions with tau.

Methods: We combined structural biology methods (NMR, CD, Fluorescence, SAXS), bioimaging (TEM, AFM), chemical kinetics (Tau aggregation) and cellular assays (seeding) to investigate the functional interactions between S100B and Tau (full length, domains, peptides) [3].

Results: We determined that S100B interacts with tau in living cells even in microtubule-destabilizing conditions. Structural analysis revealed that tau undergoes Ca²⁺-dependent dynamic interactions with S100B, notably within the aggregation repeat at the MTBR. This interaction involves contacts of tau with an interfacial cleft in S100B. S100B inhibits aggregation of tau species through effects over primary and secondary nucleation, confirmed by seeding assays and bioimaging of S100B interactions with tau oligomers/fibrils. Using tau-biosensor cells we established that S100B blocks proteopathic tau seeding [3].

POSTERS

SENESCENCE-ASSOCIATED SECRETORY PHENOTYPES REGULATES PROPAGATION OF ALPHA-SYNUCLEIN

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Aims: Accumulation of α-synuclein aggregation is hallmarks of Parkinson’s disease. A significant feature of α-synucleinopathy in Parkinson’s disease is progressive ascending propagation of this toxic protein. Recent experimental works have elucidated a cascade of propagation of α-synuclein as the underlying mechanism of progression of Parkinson’s disease. However, the exact mechanisms regarding propagation of toxic aggregates are not fully unveiled. Given that senescence has long been known as one of major risk factors of Parkinson’s disease, we hypothesized that cellular senescence could regulate the propagation of pathological α-synuclein aggregate.

Methods: Here, we established a cellular senescence model in primary cortical neurons and monitored the effects of cellular senescence on the secretion of α-synuclein.

Results: We demonstrated that neurons showed robust secretion of α-synuclein in an age-dependent manner. Inhibition of cellular senescence eased the secretion of α-synuclein in senescent neurons. Interestingly, correlative light and electron microscopy confirmed that propagating α-synuclein aggregates were present in electron-dense lysosome-like compartments. We found that the secretion of α-synuclein was highly correlated with that of β-hexosaminidase, a marker for lysosomal secretion. Ablation of lysosomal secretion showed a reduction in the secretion and propagation of α-synuclein. Furthermore, inhibition of cellular senescence nearly caused a total hindrance of the cell-to-cell propagation of α-synuclein.

Conclusions: Collectively, these results suggest that cellular senescence accelerates the propagation of α-synuclein by promoting the secretion of α-synuclein via senescence-associated lysosomal secretion.
**Aims:** Repeated mild traumatic brain injury (rmTBI) is a risk factor for Alzheimer’s disease. Prolyl oligopeptidase (PREP) and rmTBI both promote neurodegeneration. PREP negatively regulates protein phosphatase 2A (PP2A) via protein-protein interactions. Additionally, a small-molecular PREP inhibitor, KYP-2047, activates PP2A that also dephosphorylates and stabilizes Tau. PP2A participates in the clearance of toxic aggregates via increasing autophagy that TBI impairs, and PP2A’s activation reduces oxidative stress that increases following TBI. Therefore, the effects of PREP inhibitors on rmTBI-induced histological and behavioral effects were studied.

**Methods:** The effects of KYP-2047 (5 and 10 mg/kg) and a novel PREP ligand HUP-46 (10 mg/kg) were tested in C57BL/6JRccHSD mice subjected to closed-head rmTBI (3.5 m/s). Altogether 5 hits were given 24 h apart. After each hit the PREP ligands were administered (i.p.). The effects on cognition and memory were tested at 10 weeks post-rmTBI with Barnes maze. Locomotor activity was tested at 1, 6 and 11 weeks post-rmTBI. Total/phosphorylated (S262) Tau and inflammation markers (Iba1 and GFAP) were studied by immunohistochemistry and/or Western blot.

**Results:** HUP-46 counteracted the rmTBI-induced cognitive defects. A similar non-significant effect on memory was evident after KYP-2047 treatments. Unexpectedly, locomotor activity increased long-term (up to 6 weeks) in the HUP-46 and KYP-2047 treated groups after rmTBI. PREP ligands had no effects on total/phosphorylated Tau, GFAP or Iba1 levels.

**Conclusions:** PREP ligands can improve the rmTBI-induced cognitive defects in mice, though the exact mechanism is unclear. Due to long-term behavior changes, changes in plasticity following the PREP-rmTBI-interaction needs to be clarified.
Aims: Abnormal tau hyperphosphorylation and its accumulation into neurofibrillary tangles are linked to neurodegeneration in Alzheimer’s disease and similar tauopathies. Tg4510 mice are characterized by tau deposition in the brain together with cognitive impairments that resemble those seen in patients. The FDA approved drug Levetiracetam is an anticonvulsant drug known to decrease seizures in several animal models of epilepsy and recent findings show that Levetiracetam could be beneficial in the treatment of AD. The aim of this study was to test the effects of Levetiracetam diet on cognition and pathology in a mouse model of tauopathy, the Tg4510 mice.

Methods: Three-month old Tg4510 transgenic and non-transgenic littermate mice were given a Levetiracetam (40mg/kg) or normal diet for 3 months (n=10 per group). 2 weeks before tissue collection a battery of behavior tests was performed to assess cognition.

Results: Treatment with Levetiracetam did not improve cognition or activity in Tg4510 during open field, RAWM, and Ymaze. However, we found an increase in contextual fear conditioning retention upon treatment with Levetiracetam, regardless of genotype. We found that SV2A, a molecule of Levetiracetam pathway, was decreased in the hippocampus of Tg4510 mice under chow diet when compared to controls. 3 months of Levetiracetam diet restored SV2A levels in Tg4510 mice, while having no effect in non-transgenic. This was accompanied by a decrease in tau phospho serine396 in the hippocampus of Tg4510 mice treated with Levetiracetam.

Conclusions: In conclusion, we found that Levetiracetam, an FDA approved drug, partially improved cognition and reduced tau hyperphosphorylation in Tg4510 mice.
NECTANDRA RETICULATA EXTRACT DECREASES THE LEVELS OF PHOSPHORYLATION OF TAU PROTEIN AND MODULATES ASTROGLIOSIS IN THE HIPPOCAMPUS IN MODEL OF ALZHEIMER’S DISEASE (3XTG-AD)

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Aims: Evaluate the therapeutic potential of an ethanolic extract of Nectandra reticulata in the triple transgenic murine model of Alzheimer’s disease (3xTg-AD). Determine the effect of Nectandra reticulata extract on taupathy in the triple transgenic model of Alzheimer’s disease

Methods: Oral administration of the extract in a dose of 25 mg / Kg / day for 3 months to 15-month-old rodents, Morris water maze (MWM), analysis of Histopathological markers (AB, p-Tau, GFAP, APOE) by immunofluorescence and analysis with confocal microscopy.

Results: Treatment with Nectandra reticulata is a therapeutic potential as it induces changes significant molecular factors involved in pathology of AD. In the present study, evaluated the effects of a 25 mg / kg dose of the extract in cognitive and molecular patterns of the pathology presented in the 3xTg-AD model, since the study of Colombian plant extracts cobra importance given its wide wealth of secondary metabolites capable of modulating gene expressions or changes post-translations. The learning curves and the retention test did not show changes, significant between the control and treated groups, however the treated group showed a increased expression of ApoE, decreased astrogliosis and phosphorylation of Tau (AT8).

Conclusions: The dose of 25 mg / Kg / day does not lead to behavioral changes but it does induces molecular changes in proteins such as ApoE, GFAP (astrocyte marker) and the phosphorylation of Tau (AT8), indicating that the extract crosses the blood-brain barrier and is capable of activating the nuclear receptor LXR by increasing the expression of one of its targets transcriptional.
Aims: Biofluid based phosphorylated tau (p-tau) variants measurements are providing great diagnostic value in Alzheimer’s disease (AD). Despite high accuracy of cerebrospinal fluid, proceeding with this fluid has practical challenges regarding cost, access and complexity. Plasma p-tau181 and p-tau231 have well-established utility as AD biomarkers, however there is a lack of information concerning their utility in other matrices. We therefore assessed the diagnostic potential of p-tau181 and p-tau231 in serum compared with plasma in paired samples.

Methods: Using Simoa technology, p-tau231 and p-tau181 were measured in paired plasma and serum (n=15 biomarker-negative controls and n=18 biomarker-positive AD) and their diagnostic accuracies and correlations evaluated.

Results: Serum p-tau231 was increased in AD versus controls (mean=5.8pg/ml versus 1.6pg/ml; P=0.0001), AUC=88.2% (95% CI=75.3%-100%). Serum p-tau181 was increased in AD versus controls (mean=8.8pg/ml versus 4pg/ml; P=0.0001), AUC=89.6% (95% CI=78.5%-100%). Plasma p-tau231 was increased in AD versus controls (mean=11.4pg/ml versus 4.9pg/ml; P=0.0001), AUC=90.2% (95% CI=79%-100%). Plasma p-tau181 was increased in AD versus controls (mean=9.2pg/ml versus 4.7pg/ml; P=0.0003), AUC=87.5% (95% CI=71.1%-98.9%). We observed significant (P=<0.0001) correlations (r=0.78-0.92) between all blood biomarkers.

Conclusions: Our results show that serum p-tau181 and p-tau231 can effectively discriminate biomarker-positive AD from biomarker-negative controls in this relatively small cohort. Serum p-tau231 and ptau181 tend to perform similarly to plasma measurements, but the findings need replication in larger cohorts. This finding expands p-tau181 and p-tau231 analyses to research cohorts and hospital systems that prefer serum to other blood matrices.
Aims: Tau protein aggregation is a hallmark of several neurodegenerative disorders, including Alzheimer's disease (AD). The search for a pathology modifier therapy continues and passive immunotherapy to prevent Tau aggregation is a hot-topic. In this study, we aim to generate and characterize novel high affinity monoclonal antibodies (mAbs) as therapeutic candidates to combat AD.

Methods: Two novel anti-Tau mAbs, 9H6 and 11E12 were generated by immunizing mice with human Tau phosphorylated on pathology-relevant epitopes isolated from humanized yeast models. The mAb epitopes were determined using a library of overlapping synthetic peptides (Pepscan, Lelystad, Netherlands). Their affinity for recombinant and human brain Tau was determined in ELISA. Both mAbs were tested together with the previously generated antibodies ADx215, ADx201, 18F12, 15A10, 16B12 and 20G10 in a cellular Tau seeding assay for their capacity to inhibit Tau aggregation when using human AD-brain Tau seeds.

Results: The two novel anti-Tau mAbs have a high affinity to human Tau. The mAb 11E12 has a linear continuous epitope, while 9H6 has a discontinuous epitope with three segments in the proline-rich domain. In the cellular Tau seeding assay, the therapeutic candidate mAbs decreased seeding efficiency of AD-brain Tau seeds between 24% and 50%.

Conclusions: These preliminary results on Tau seeding inhibition support the use of anti-Tau mAbs as therapeutic candidates for AD and further in vivo studies will be conducted.
DEVELOPMENT OF ANTI-TAU SINGLE-CHAIN VARIABLE FRAGMENTS FOR USE AS INTRABODIES

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Aims: Over the last decade, there has been a growing interest in intrabodies and their therapeutic potential. Intrabodies are recombinant antibodies or antibody fragments engineered to be expressed inside the cell to target their antigen intracellularly. Intracellular expression of functional antibody fragments can be challenging, as the reducing environment does not favor the formation of disulfide bonds, which can be important for correct folding and activity. Here, we have developed anti-Tau scFvs that have the potential to be used as intrabodies to target Tau pathology in Alzheimer’s Disease (AD).

Methods: A group of well characterized anti-Tau monoclonal antibodies (mAbs) were converted to scFvs and expressed in the cytoplasm of HEK293 cells. Retention of Tau-binding was evaluated using direct ELISA assays against full length recombinant human Tau protein and AD brain-derived paired helical fragments. Moreover, ability to bind intracellular Tau was evaluated by scFv co-expression with full length recombinant human Tau coupled to a nuclear localization signal in the cytoplasm of HEK293 cells, followed by immunocytochemistry.

Results: ELISA results showed that we have constructed scFvs that retain Tau binding, both against monomeric and aggregated Tau. Additionally, we also demonstrate that these scFvs are functional in the reducing environment of the cytoplasm.

Conclusions: These constructs will be further assessed for their potency to reduce tau aggregation in in vitro and in vivo systems.
Aims: Alzheimer’s Disease (AD) is a devastating neurodegenerative disease characterized by memory impairment and cognitive defects. Since tau hyper-phosphorylation and its consequent imbalance are clearly involved in its aggregation, protein kinases represent new targets of great interest. Serum and glucocorticoid-regulated kinase 1 (SGK1) is a novel kinase able to phosphorylate tau whose role in AD is still undeciphered. Goals: SGK1 expression patterns in different AD models, discovery of new potent SGK1 inhibitors and evaluation of their potential anti-tau profile.

Methods: In this work, SGK1 expression was characterized in different human and mouse AD models by means of quantitative polymerase chain reaction (qPCR). Subsequently, a high-throughput ligand and structure-based virtual screening with the EU-OPENSCREEN library (100,000 structurally diverse drug-like compounds) was performed. Finally, a viability assay against the toxic effect of okadaic acid was carried out in SH-SY5Y cells with those inhibitors obtained from the previous step.

Results: An increasing tendency in the expression of SGK1 can be observed, mainly in human hippocampus and lymphocytes samples. The successful combination of both ligand and structure-based methods led to the discovery of several potent and structurally diverse SGK1 inhibitors with IC50 values ranging from low micromolar to low nanomolar. Finally, these compounds were able to counteract the toxic effect of okadaic acid, prompting the recovery of the cell viability.

Conclusions: The obtained results points SGK1 as an interesting target in race for an AD treatment, being these new inhibitors a privileged starting point to develop a pharmacological tool against this disease.
DEVELOPMENT AND NEUROBIOLOGICAL EVALUATION OF NEW BENZIMIDAZOLE AND INDOLE DERIVATIVES AS POTENTIAL MULTI-TARGET DRUGS FOR THE TREATMENT OF PARKINSON’S DISEASE

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Aims: The aim of the study was the synthesis of new compounds with multi-target activity as potential anti-parkinsonian agents and the evaluation of their neurobiological properties including neurotoxicity, neuroprotection, MAO-B inhibition and BBB permeability, and antioxidant effect.

Methods: Series of benzimidazole arylhydrazones containing a propargyl moiety as well as methoxy and hydroxy substituted derivatives of the indole propionic acid and 5-methoxyindole were synthesized. Initially the neurotoxicological potential of the compounds was evaluated and the least toxic were selected for neuroprotective properties studies on two in vitro models of H2O2-induced oxidative stress in neuroblastoma SH-SY5Y cells and 6-hydroxydopamine (6-OHDA) induced neurotoxicity in rat brain synaptosomes and the cell viability, synaptosomal viability, and intra-synaptosomal content of GSH were determined. The monoamine oxidase-B inhibitory potential was studied on human recombinant MAO-B enzyme. The permeability of an endothelial monolayer of b.End3 cell line upon treatment with selected compounds was used as an in vitro static monolayer BBB model. For further investigation of the antioxidant properties, the capability of the derivatives to decrease the level of molecular damage of biologically important molecules upon ferrous iron-induced oxidative molecular damage was studied.

Results: The obtained results reveal that the tested compounds possess good safety profile and demonstrate significant neuroprotective effect in combination with MAO-B inhibiting properties higher than the referent rasagiline and melatonin. Furthermore, they are capable to inhibit the lipid peroxidation.

Conclusions: Our preliminary studies suggest that the benzimidazole and indole derivatives containing hydroxy and methoxy arylhydrazone functionalities show promising potential for the development of new pharmaceuticals for the treatment of PD.
Aims: The high value for Alzheimer’s Disease and other taupathies of a small molecule treatment that can alter disease course is widely acknowledged. We aim to investigate such a therapeutic by leveraging our small molecule CNS aggregated protein discovery platform.

Methods: Our small molecule discovery platform consists of our proprietary small molecule collection, unique PET imaging biomarkers and cryoEM structures. Our proprietary collection of CNS-focused aggregated protein binding agents, previously developed by APRINOIA to map the structure-activity relationship of its tau PET tracer programs, was screened for tau binding and further interrogated for other desirable properties such as selectivity and oral availability. Further efforts derived guidance from the cryoEM structure of our lead compound bound to Tau aggregates. These efforts resulted in our small molecule lead compound APN-1808. Using rTg4510 mice, which over express 4R tau, we first investigated dose-response by using in-vivo PET imaging to measure percent occupancy of APN-1808. Upon selecting an appropriate dose range, we engaged in 90 day, twice a day dosing of APN-1808 in rTg4510 mice.

Results: Mice dosed with APN-1808 showed improvements both biochemically and phenotypically over vehicle controls. Improvements included increase in synaptic protein level, reduction in overall tau burden, reduction in weight loss and other behavioral observations.

Conclusions: Several readouts showed improvement in the dosed groups compared to controls, including increase of synapse level, reduction of weight loss and reduction of total tau. Further investigations are ongoing.
Aims: Microglia are the principal immune cells in the central nervous system, serving as the modulators of brain homeostasis. By rapidly responding to noxious stimuli, activated microglia could release extracellular vesicles (EVs) carrying various pro-inflammatory cytokines. However, the molecules critical for regulating the EV production from microglia are yet to be understood.

Methods: Here, we have established a murine microglial cell model to monitor the extracellular vesicle secretion via measuring the fluorescence signal of tdTomato, which was linked with tetraspanin CD63. Stimulation of tdTomato+ cells with ATP induces rapid secretion of EVs and reduction in tdTomato intensity, which is a readout of the EV secretion. We have generated GFP+ tdTomato+ cell library after infection of lentiviral library expressing turboGFP and barcoded short-hairpin RNA (shRNA) library for genome-wide screening.

Results: After sorting of GFP+ tdTomato^high and GFP+ tdTomato^low cells upon ATP stimulation combined with next generation sequencing, we identified 1353 host genes with z-score higher than 2.5, which were resistant to the ATP-induced EV exocytosis. Gene ontology analysis identified the enrichment of the integral component of membrane and plasma membrane component, and their involvement in the cell-to-cell communication. Individual validation of the top hits identified that the Mcfd2, Sepp1 and Sdc1 critically regulate EV secretion from murine microglia. Finally, siRNA-based silencing of these three genes suppressed the lipopolysaccharide and ATP-induced inflammasome activation as determined by interleukin-1β release from primary cultured murine microglia.

Conclusions: Our genome-wide screening study identified three novel candidate genes for microglial EV secretion, which are potential therapeutic target of neuroinflammatory disorders.
**Aims:** Intraneuronal tau protein aggregates are a hallmark of many neurodegenerative disorders, including Alzheimer’s Disease. Anti-tau antibodies show therapeutic promise in pre-clinical models, however their exact mechanism of action remains elusive and efficacy is limited by access to the CNS and to intracellular tau aggregates. To better engage these aggregates and target them towards a known degradation pathway we have utilised intracellularly expressed protein constructs consisting of a protein recognition nanobody domain fused to the E3 ubiquitin ligase domain of TRIM21 (miniTRIM-away).

**Methods:** miniTRIM-away constructs packaged into adeno-associated viral (AAV) vectors were used to transduce cells in various models of tau pathology including: cell lines stably expressing soluble or aggregated tau-venus, primary neuronal culture, and murine organotypic hippocampal slice cultures. For in vivo proof of concept, AAV packaged GFP targeting miniTRIM-away constructs were injected into the hippocampi of transgenic H2B-GFP mice.

**Results:** Using miniTRIM-away constructs that bind to GFP or tau, we demonstrate that both soluble and aggregated tau-venus can be degraded in cell-based assays. We demonstrate that miniTRIM-away can successfully and specifically degrade target proteins in the brain following stereotaxic injection of AAV to the hippocampus.

**Conclusions:** MiniTRIM-away provides a route to the selective degradation of target proteins. Both soluble and aggregated tau species are liable to degradation. Future developments will optimise this degradative effect and assess its capability to reduce the burden of tau pathology.
A NOVEL THERAPEUTIC AGENT FOR ALZHEIMER'S DISEASE USING AMYLOID BETA-SPECIFIC HUMAN REGULATORY T CELLS TO CONTROL MICROGLIA ACTIVATION IN 3XTG MOUSE BRAIN.

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Aims: Alzheimer's disease (AD) is a common form of dementia, accompanied by loss of memory, impairment in learning and thinking, depression, and other symptoms. Neuroinflammation is a major feature of Alzheimer's disease pathology and is closely related to this disease's amyloid and tau pathology. Such brain neuroinflammation is regulated by microglia, which constitute the innate immune system of the central nervous system. Recently, the therapeutic effect of a specific subpopulation of T cells (CD4+CD25+Foxp3+ regulatory T cells, Tregs) that can suppress excessive inflammation in various immune and inflammatory disorders has been reported. Here we confirm whether expanded amyloid-beta (Aβ)-specific Tregs with bvPLA2 in vitro (designated Ag-hTreg, VT301) exhibits efficacy as a therapeutic agent for neuro-inflammation.

Methods: Expanded human Ag-Tregs were intrathecally administrated to 10-month-old 3xTG mice. Two-month after intrathecal administration, we performed and tested cognitive recovery, expression of microglia markers (types M1 or M2 and DAM) and deposition of tau protein by behavior test, RT-qPCR, WB, and ICC.

Results: The significant neuro inflammatory inhibitory effect promoted by Ag-Tregs modulated microglia properties, which reduced the risk of AD through microglia. In addition, the expression of p-Tau protein in the brain of Treg-treated AD mice was lowered similarly to that of WT

Conclusions: Therefore, our finding opens up the possibility of the clinical application using Treg as a cell therapy for Alzheimer's disease or microglia-related neuroinflammatory diseases.
Aims: While many studies have revealed that microglia contribute to Alzheimer’s disease (AD) pathogenesis, the impact of depleting and repopulating these cells is less clear. In this study, we sought to characterize major pathological hallmarks of AD and changes in the microglial transcriptome following a PLX-based depletion and repopulation paradigm.

Methods: 22 to 24-month-old, male 3xTg mice were placed on a PLX5622 diet for 2 weeks to deplete microglia. Mice were returned to control chow for 4 weeks, after which one hemisphere was collected for immunohistochemistry (IHC). Hippocampi from 3-5 mice were pooled for single-cell RNAseq (scRNAseq) of CD45

Results: Repopulating microglia did not influence amyloid pathology based on IHC or ELISA measurements but, unexpectedly, increased pT205 Tau phosphorylation. This was associated with decreased P2RY12 but not TMEM119 expression. Furthermore, we identified several well-established, as well as novel clusters of microglia through scRNAseq. In particular, we observed increased expression of Cxcl13 in repopulated microglia. Using RNAScope, we discovered increased Cxcl13 RNA in the CA1, but not in the subiculum.

Conclusions: We conclude that repopulating microglia in 3xTg mice did not affect amyloid pathology but increased Tau phosphorylation. This was correlated with increased microglial expression of Cxcl13, the effect of which is relatively understudied in AD. (Funding sources: R01 AG030149, R56 AG066397, Del Monte Neuroscience Institute Pilot Program, F99NS108486-02S1, AARG-NTF-19-619116)
POSTERS

DETECTION OF PRODROMAL EARLY PHENOTYPES AND POTENTIAL THERAPEUTIC WINDOW IN A MODEL OF TAUOPATHY

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Aims: Characterize the time course of tau pathology and the onset of early behavioral and molecular phenotypes in a model of tauopathy, to establish a reliable window for therapeutic intervention.

Methods: The htau mouse model of tauopathy, which bears an abnormal 3R:4R tau isoforms imbalance, was analyzed between 2 and 12 months-old through a battery of behavioral tests (including sensory, motor and cognitive tasks) followed by electrophysiological and immunochemical analysis. Viral vectors carrying molecules that modulate 3R:4R isoforms by trans-splicing were injected either in the striatum or PFC of hTau mice either at 3 or 6 months old and phenotypic rescue was assessed.

Results: Htau mice display cognitive deficits, anxiety phenotypes, motor impulsivity and loss of behavioral inhibition, which correlate with abnormal tau 3R:4R ratio in the prefrontal cortex and the striatum. Local modulation of the 3R:4R ratio by trans-splicing in the striatum improved some of the htau phenotypes.

Conclusions: Our results suggest that tau isoforms imbalance underlie early phenotypes before the onset of cognitive deficits, that can be identified to determine the optimal window for therapeutic strategies.
Aims: Objectives: PD patients manifest a variety of ancillary problems during the course of the disease (e.g., cognitive dysfunction, dementia, psychosis, autonomic dysfunction, sleep-disorders, depression, behavioral disorders and constipation), some of which are exacerbated by L-dopa therapy. L-dopa/benserazide or L-dopa/carbidopa combinations are widely used for the treatment of Parkinson’s disease to reduce peripheral conversion of L-dopa to dopamine before it reaches the CNS. Tryptophan hydroxylase (TPH) catalyzes the first and rate-limiting step in the biosynthesis of the neurotransmitter serotonin. TPH1 plays the same role for peripheral serotonin biosynthesis. A decrease in the activity of the TPH2 enzyme in the CNS is associated with depression, while TPH2 in intestinal serotonergic neurons controls intestinal motility.

Methods: Human TPH2 and TPH1 were expressed in E. coli using an auto-induction media and the proteins were purified. Enzyme activities were measured with a radio-enzymatic 3H2O release assay in the presence of benserazide, carbidopa, L-dopa and dopamine at 20 µM. Subsequently, IC50 values were determined from dose effect curves.

Results: In this study, benserazide, carbidopa, L-DOPA and dopamine (at 20 µM) were found to decrease TPH2 activity by 76.4%, 15.2%, 0 and 13.5%; and to decrease TPH1 activity by 71.2%, 10%, 47.6% and 25.4%. The IC50 values for TPH2 were also measured as 7.6, 310, 339 and 123µM, respectively.

Conclusions: Administration of the combination of L-dopa/benserazide or carbidopa may cause problems of peripheral autonomic dysfunction and enteric nervous system inhibition following serotonin depletion (manifested as constipation problems seen in Parkinson’s treatment).
Aims: As a follow-up to preclinical studies demonstrating the role of senescence in Alzheimer's disease, we conducted a pilot study to collect preliminary data on the safety and efficacy of a senolytic regimen (dasatinib plus quercetin) for attenuating the progression of clinical Alzheimer’s disease.

Methods: Five individuals over 65 with early-stage Alzheimer’s disease were enrolled in an open-label senolytic therapy trial. Participants were asked to take 100 mg of dasatinib plus 1,000 mg of quercetin daily for two consecutive days followed by a 13-15 day no-medication period for six total cycles over a 12-week period. Pre- and post-treatment assessments included physical and neurological examination, cognitive assessments, brain MRI, and blood and cerebrospinal fluid collection. Adverse event and safety monitoring were performed throughout the study.

Results: No serious adverse events occurred. A total of seven mild to moderate adverse events were recorded during the course of the study including gastrointestinal distress (two occurrences), emesis (one occurrence), urinary tract infection (one occurrence), hematuria (one occurrence), hypoglycemia (one occurrence), and a fall (one occurrence). All adverse events fully resolved and no study drop outs occurred.

Conclusions: Preliminary data suggests that short-term, intermittent senolytic therapy is well-tolerated among older adults with Alzheimer’s disease. A larger, randomized placebo-controlled study is necessary for further evaluation of safety and efficacy.
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FINAL DATA FROM PHASE 1 SAD TRIAL OF PNT001, A MONOCLONAL ANTIBODY UNIQUELY RECOGNIZING CIS-PT231 TAU FOR TREATMENT OF TAUOPATHIES

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Aims: Pinteon Therapeutics has generated a humanized monoclonal antibody, PNT001, that uniquely recognizes cis-pT231 tau with high affinity and selectivity. Cis-pT231 tau, present selectively in the brains of tauopathy patients, is a potent driver of tau-related neurodegeneration. In preclinical models, PNT001 reduces the spread of tau pathology, neuroinflammation, and neurodegeneration. It improves synaptic and functional endpoints, and depletes aggregation-competent tau from AD and PSP brains. A Phase 1 trial in healthy volunteers is complete.

Methods: The single-dose, double-blind, placebo-controlled dose cohort escalation trial employing dose levels from 33mg-4000mg evaluated safety, tolerability, CSF and serum pharmacokinetic (PK), anti-drug antibodies and biomarker data in healthy volunteers over 16 weeks. Dose escalation was determined by an external, independent safety board.

Results: Thirty-six (36) subjects received PNT001. There were three related non-serious adverse events, each Grade 1, at the lowest doses, that resolved without sequelae. No maximum tolerated dose was identified. There were no premature discontinuations, dose reductions or interruptions due to treatment related adverse events. One unrelated serious adverse event occurred in a placebo subject with an undisclosed medical condition. Doses of 900mg - 4000 produced CSF concentrations exceeding the binding affinity constant of PNT001 for cis-pT231 tau indicating CSF concentrations sufficient for target engagement. PK was as expected for a monoclonal antibody. The terminal half life ranged from 23.8-33.8 days and CSF exposures were dose proportional.

Conclusions: PNT001 at all dose levels was well tolerated, including those doses expected to produce target engagement, supporting multiple ascending dose trials in patients with neurodegenerative tauopathies.
STRUCTURE BASED DISCOVERY OF THIAZOLIDIN-4-ONE ANALOGUES AS ATP NON-COMPETITIVE GSK-3β INHIBITORS

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Aims: One of the characteristic hallmarks of Alzheimer’s disease (AD) is the accumulation of neurofibrillary tangles (NFT). This protein is produced by hyperphosphorylation of tau protein by kinases. Among all kinases Glycogen synthase kinase-3β (GSK-3β) catalyzes the rate-limiting step of the NFT generation, therefore GSK-3β has been proposed as a promising target for the treatment of AD. Inhibition of GSK-3β hyperphosphorylation activity would halt the formation of NFT at the very beginning, hence serving as a potential target for Alzheimer’s disease.

Methods: In this study, we performed structure-based virtual screening of Chembridge library of 1524 derivatives of thiazolidine-4-one against GSK-3β for calculating dock score using Glide and MM-GBSA dG calculation using Prime module of Maestro (Schrodinger) to identify novel GSK-3β inhibitors. On basis of dock score, MM-GBSA dG, protein-ligand interactions, and structural features 10 compounds were selected for further protein kinase assay against GSK-3β, DYRK1A, CDK-2, CDK-5, CDK-9, and CLK-1 and ATP competitive assay was performed to determine the mechanism of inhibition.

Results: New GSK-3β inhibitors were identified using a structure-based screening, in-vitro assay, and ATM competition assay. Through these studies, 6190954, 7482634, 6209717, and 5987896 emerged as potent hits with an IC₅₀ range of 1.23 – 2.34 µM. During disease kinase profiling, these all have emerged as selective GSK-3β inhibitors with ATP non-competitive inhibition of GSK-3β.

Conclusions: Novel thiazolidin-4-one analogues were identified as ATP non-competitive and selective GSK-3β inhibitors, which may serve as a tool in the future for designing new strategies for selective GSK-3β inhibition.
Aims: Alzheimer’s Disease (AD) is one of the significant diseases of the aging population and affects Central Nervous System dominantly. Overactivity of Glycogen synthase kinase-3β (GSK-3β) causes tau's hyperphosphorylation, leading to microtubule destabilization, neurofibrillary tangles formation, senile plaque formation/deposition, which are the major hallmarks of AD, hence making GSK-3β an attractive target for AD. Although GSK-3β is an attractive target for AD, there is no single GSK-3β inhibitor in clinical trials for AD. Therefore, the development of GSK-3β inhibitors is a prompting challenge and is the need of the hour for AD treatment by disease-modifying approach.

Methods: In the current study, 50 derivatives of tetrahydropyrimidine-5-carboxamide were synthesized and investigated for in-vitro kinase inhibitory activity against GSK-3β and other related kinases. Molecular modeling studies were performed on the synthesized compounds to get an insight into the protein-ligand interaction. ATP-competition assay was performed for determining the mechanism of action of synthesized molecules.

Results: Here we report the synthesis of 50 compounds with good to excellent yields. Synthesized compounds showed promising results; compound RM-SU-J-124 showed IC_{50} of 6.06 µM against GSK-3β and showed selectivity over other related kinases. Kinetic experiments revealed that compounds were inhibiting GSK-3β in an ATP-competitive manner.

Conclusions: We successfully identified tetrahydropyrimidine-5-carboxamide derivatives as selective and ATP-Competitive GSK-3β inhibitors. These results will encourage the exploration of tetrahydropyrimidine-5-carboxamide as a valuable drug lead for the treatment of AD.
Aims: Neuronal accumulation of neurofibrillary tangles (NFT) is a major pathological hallmark of Alzheimer’s disease (AD). NFT is produced by hyperphosphorylation of tau protein by kinases like CDK-5, DYRK1A, CK-1δ, GSK-3β, etc. Inhibition of hyperphosphorylation activity of these kinases would halt the formation of NFT at the very beginning, hence serves as a potential target for inhibition of progression of Alzheimer’s disease.

Methods: Performed structure-based virtual screening of Chembridge library of 2706 analogues of pyrazolo[3,4d]pyrimidine-4-amines against CDK-5, DYRK1A, and CK-1δ for calculating dock score using Glide and MM-GBSA dG calculation using Prime module of Maestro (Schrodinger) to identify novel GSK-3β inhibitors. On basis of targeting more than one kinase, 18 compounds were selected for further protein kinase assay against CK-1δ, DYRK1A, CDK-5, GSK-3β, Haspin, CLK-1, and in-vivo studies in C. elegans models.

Results: Through these studies 91399287, 60465099, and 88882793 emerged as potent multitargeted hits with an IC₅₀ range of 0.11 – 4.7 µM. Also, during disease kinase profiling, 96328641 and 60421049 emerged as selective CLK-1 inhibitors and 40297912 as selective CK1δ inhibitor respectively.

Conclusions: These identified novel pyrazolo[3,4d]pyrimidine-4-amines analogues may serve in future as a tool for multitargeted kinase inhibition and developing new strategies for AD treatment.
Aims: Alzheimer’s Disease (AD) is one of the prominent diseases in the elderly population. Overactivity of Glycogen synthase kinase-3β (GSK-3β) causes tau’s hyperphosphorylation, leading to microtubule destabilization, neurofibrillary tangles formation, hence making GSK-3β an attractive target for AD. Development of GSK-3β inhibitors is a prompting challenge and is the need of the hour for AD treatment by a disease-modifying approach.

Methods: Here we designed and synthesized 26 derivatives of 4-phenyl-tetrahydropyrimidines using ethanol as solvent and microwave-assisted green chemistry approach. Synthesized compounds were investigated for in-vitro kinase inhibitory activity against GSK-3β and other related kinases for calculating IC₅₀ and selectivity profiling. ATP-competition assay was performed for determining the mechanism of inhibition of synthesized molecules. Potent hits obtained from in-vitro kinase profiling were further subjected to in-vivo assay in C. elegans.

Results: Synthesized compounds showed promising results; compounds RM-SU-71 and RM-SU-68 showed IC₅₀ of 0.90 and 1.69 µM respectively against GSK-3β and showed selectivity over other related kinases. Kinetic experiments revealed that compounds were inhibiting GSK-3β in an ATP-competitive manner.

Conclusions: These resulted potent hits will encourage exploration of more 4-phenyl-tetrahydropyrimidine for the development of valuable lead candidates for the treatment of AD.
Aims: BACE1 has become an important therapeutic target in AD. However, many BACE1 inhibitors have been developed as therapeutic agents to overcome AD by disturbing BACE1 activity and these drugs are anticipated to decrease production of Aβ, but commercialization has failed. The main reason, why BACE1 inhibitors clinical trial had fail, may stem from function of BACE1 plays in a healthy brain. Here, we established a new strategy that modulates expression rather than inhibiting BACE1 to solve these side effects, which disturb the normal function of BACE1.

Methods: In order to intuitively and quickly select a drug that regulates BACE1 promoter activity, we established a light-emitting system that can directly confirm the activity of the human BACE1 promoter. By inserting a human BACE1 promoter sequence into a psDNA3.1-EGFP vector, which have a green fluorescent protein (GFP), we constructed a plasmid(pHB1PG) expressing GFP when the BACE1 promoter is activated.

Results: We have screened FDA-approved drug library to identify drugs which reduce promoter activity of BACE1. Here, we have found a new candidate drug 6-Thioguanosine (6-TG), which reduce promoter activity, mRNA and protein levels of BACE1 in different types of neuronal cell. We have confirmed that 6-TG decrease BACE1 gene expression, prevent accumulation of Aβ and improve cognitive function in APP/PS1 transgenic mice.

Conclusions: In conclusion we found that 6-TG reduces BACE1 expression in vitro as well as in vivo, which means that 6-TG can mitigate Alzheimer’s disease neuropathy and be developed as a therapeutic agent for Alzheimer’s disease.
ASSOCIATION BETWEEN SPEECH CHARACTERISTICS AND CORTICAL [18F]GTP1 TAU PET TAU LEVELS IN PRODROMAL-TO-MILD ALZHEIMER’S DISEASE

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Aims: Speech changes in Alzheimer’s disease (AD) are potential early indicators of disease, but validation against established AD biomarkers is lacking. We sought to determine if cerebral tau accumulation measured by tau PET imaging is associated with speech characteristics in prodromal-to-mild AD.

Methods: Baseline data from a subset (N=83) of right-handed English-speaking participants in the Tauriel trial of semorinemab in prodromal-to-mild AD were analyzed. Speech samples recorded from Clinical Dementia Rating (CDR) administrations were analyzed using the Winterlight speech processing pipeline, generating over 500 acoustic and linguistic speech variables. Pearson correlations were computed to determine cross-sectional associations between speech features and [¹⁸F]GTP1 tau PET SUVR values in whole cortical grey and other regions of interest (ROIs).

Results: Significant correlations were found between [¹⁸F]GTP1 SUVR and speech characteristics. The strongest association was between the usage of the filled pause “um” and [¹⁸F]GTP1 SUVR in whole cortical grey (r=0.47, p<0.01) and select language-related ROIs. Other associations with increased [¹⁸F]GTP1 SUVR included increased use of more frequent words, lower vocabulary richness and reduced speech rate (r’s=0.28-0.36).

Conclusions: Increased use of filled pauses was associated with higher cortical tau PET signal in AD. Using filled pauses more frequently may indicate word finding difficulty and/or memory impairment, which may represent increased underlying AD pathology. Likewise, the association of other speech patterns with tau PET indices, including simpler vocabulary and slower speech rate, are consistent with the hypothesis that increased cortical tau deposition may drive altered speech patterns associated with disease progression.
Aims: According to WHO reports, Alzheimer’s disease (AD) is the most common form of senile dementia occurring in later life and is a major cause of disability and death in the elderly. The aim of this presentation is to describe the potential effects of grape seed extract (GSE) in treatment of Alzheimer’s disease.

Methods: Different methods have been developed for the study of AD in animals. The most commonly used neurotoxins included excitatory amino acid neurotransmitters such as glutamate. The acute neurodegenerative effect of amyloid beta and amyloid cores from the brains of AD patients was demonstrated in vivo. GSE is able to alleviate the consequences of the AD in animal models and in human.

Results: A group of compounds found in grape GSE plaque formation and resulting cognitive impairment in an animal model of Alzheimer’s disease. Polyphenols extracted from grape seeds are able to inhibit amyloid-beta (Ab) aggregation, reduce Ab production and protect against Ab neurotoxicity in vitro. Significantly inhibit amyloid β-protein aggregation into high-molecular-weight oligomers in vitro. When orally administered to Tg2576 mice, this polyphenolic preparation significantly attenuates AD-type cognitive deterioration coincidentally with reduced HMW soluble oligomeric Aβ in the brain.

Conclusions: Different studies suggest that GSE-derived polyphenolics constituents may be useful agents to prevent or treat AD. We may recommend the oral administration of GSE preparations in patients with AD.
IMPROVING ALZHEIMER’S DISEASE CLASSIFICATION FROM BRAIN MRI WITH AN ATTENTION-GUIDED GENERATIVE ADVERSARIAL NETWORK AND TRANSFER LEARNING

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Aims: Millions of people all over the world are impacted by Alzheimer’s disease leading to permanent memory loss and gradual neurodegeneration. Clinicians are now focussing on objective tests along with cognitive tests for clinical trial patient stratifications. Biomarkers like MRI scans are widely available and are very less invasive and hence AD classification from MRI scans using deep learning methods are becoming predominant. However, MRI scans from different scanners and protocols differ from each other and hamper the AD classification performance. Here we have built upon the work named in figure below [1] and performed MRI scan domain adaptation and AD classification for the same.

Alzheimer’s Disease Classification Accuracy is Improved by MRI Harmonization based on Attention-Guided Generative Adversarial Networks

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Methods: We performed MRI scan domain adaptation for 4 different sources namely UKBB, ADNI, AIBL and OASIS using an attention guided generative adversarial network. For the remaining sources we followed domain adaptation as in [1]. Following which we tried two methodologies for improving Alzheimer’s classification performance as compared to [1]. Firstly, using an attention based 2D Alexnet (Fig 2) and secondly, transfer learning using a pre-trained model on sex classification task.
**Results:** For domain adaptation from UKBB to ADNI we achieved average LSH scores of 0.88 suggesting preservation of style similarity after domain adaptation. For AD classification we improved our accuracy as shown in figure below.

<table>
<thead>
<tr>
<th>Model</th>
<th>Non harmonized scan accuracy</th>
<th>Harmonized scan accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Default Alexnet model</td>
<td>74.05%</td>
<td>83.67%</td>
</tr>
<tr>
<td>Attention based 2D Alexnet</td>
<td>75.51%</td>
<td>84.69%</td>
</tr>
<tr>
<td>Transfer learning based model</td>
<td>74.63%</td>
<td>83.55%</td>
</tr>
</tbody>
</table>

**Conclusions:** Differences in MRI scans from various sources make preliminary AD detection harder and domain adaptation is an efficient way to improve classification accuracy. In this work we also observed how attention mechanisms and transfer learning can further improve performance.
UTILITY EVALUATION OF A TAU TRACER, [18F]PI-2620, IN ALZHEIMER’S DISEASE (AD) AND NON-AD TAUOPATHIES

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Aims: Tau aggregates are pathological features of neurodegenerative diseases including Alzheimer’s disease (AD). Preclinically, a tau tracer, [18F]PI-2620, was indicated to have desirable properties for visualizing tau accumulation in both AD and non-AD tauopathies. The aim of this study is to assess the ability of [18F]PI-2620 to detect regional tau burden in AD and non-AD tauopathies.

Methods: We recruited healthy volunteers (N=7), and patients with AD (N=8), progressive supranuclear palsy (PSP) (N=3), corticobasal syndrome (CBS) (N=2), pathologically-confirmed corticobasal degeneration (CBD) (N=1). [18F]PI-2620 tau PET was performed, and regional accumulation was assessed by calculating standardized uptake value ratios (SUVRs). Furthermore, postmortem analysis for radio-pathological correlation was performed in one CBD patient. In addition, in one PSP patient, we performed head-to-head comparison of [18F]PI-2620 and [18F]PM-PBB3, another tau tracer for possibly visualizing 4-repeat (4R) tauopathies.

Results: In AD, focal retention of [18F]PI-2620 was apparent in temporal, parietal, and cingulate cortex. SUVR analysis revealed that more prominent uptake in globus pallidus and midbrain was observed in PSP, CBS, and CBD in comparison to AD, whereas such discrepancies were not apparent in other regions with expected 4R-tau accumulation. In CBD, radio-pathological analysis failed to show a strong correlation between region-matched [18F]PI-2620 retention in vivo and postmortem burden of tau. In PSP, a head-to-head comparison of [18F]PI-2620 and [18F]PM-PBB3 revealed different retention patterns.
Conclusions: Although \[^{18}F\]PI-2620 can detect tau burden in AD, in vivo retention doesn’t correlate with expected 4R-tau burden in non-AD tauopathies. \[^{18}F\]PI-2620 may have limited utility for reliable detection of 4R-tau pathology.
POSTERS

DECREASED SLOW-WAVE SLEEP IS ASSOCIATED WITH GREATER PATHOLOGIC TAU ACCUMULATION IN PRECLINICAL PHASES OF ALZHEIMER’S DISEASE

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Aims: The accumulation of β-amyloid plaques and development of tau neurofibrillary tangles are core neuropathological features of Alzheimer’s disease (AD). The accumulation of these proteinopathies reflects a complex interplay of Aβ and tau production, processing, and clearance. Prior work suggests sleep may be critically important to tau and Aβ production and clearance. Accordingly, we examined associations between sleep macroarchitecture and regional tau PET (18F-flortaucipir;FTP) and amyloid PET (11C-Pittsburgh Compound B;PiB) in a sample of clinically-unimpaired adults at risk of developing AD.

Methods: At-home polysomnography (PSG; Compumedics Somte), FTP, and PiB PET were assessed in 40 clinically-normal older adults (mean age 73.3 years).

Results: Controlling for age and sex, we observed that less slow-wave (SWS; p = 0.009) and greater Stage 1 (N1, p = 0.001) sleep were associated with greater tau burden in the inferior temporal cortex and other adjacent regions within the temporal lobe. Greater rapid eye movement (REM) sleep had an association with lesser tau PET signal, but this association did not survive correction for age. Intriguingly, no associations between amyloid PET and sleep macroarchitecture were observed, and associations between tau accumulation and both SWS or N1 were independent of β-amyloid burden as measured by PiB PET.

Conclusions: These results suggest that decreased ability to transition to slow-wave sleep from shallower stages of sleep may accelerate tau accumulation in areas of the temporal lobe known to be affected in Alzheimer’s disease. Strategies to increase slow-wave sleep may be beneficial to reduce tau accumulation alone or in combination with amyloid-reducing approaches.
POSTERS

PROGRESS IN DEVELOPING A LIGHT-STABLE 4R TAU PET IMAGING AGENT: APN-1701 FIH

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Aims: The need for a light stable 4R tau PET tracer for use in diverse tauopathies is widely appreciated. We aimed to assess the initial human imaging profile of [18F]APN-1701 as such a tracer and to compare its imaging profile to the well-established but light-sensitive tracer [18F]-APN-1607 that can detect 4R aggregates as well as 3R and mixed 3R/4R aggregates. [18F]APN-1607 has progressed to a multicenter Phase 2 clinical trial for Alzheimer’s disease (AD); therefore, prior imaging data with it was considered valuable for comparison.

Methods: One cognitively normal (CN) subject (male, 73yo) and one AD subject (female, 58 yo), who had undergone prior amyloid profiling using [18F]Florbetapir PET and subsequent imaging with [18F]APN-1607 in a proof-of-concept study and a test-retest study, respectively, participated in the current study. Dynamic imaging with [18F]APN-1701 was performed over 180 min to obtain kinetic data.

Results: Overall, for both subjects [18F]APN-1701 generally matched [18F]APN-1607 findings, i.e., peak SUV values reached similar levels. The CN subject, who was amyloid negative, had slight, uniform cortical [18F]-APN-1701 uptake and more substantial uptake in the pallidum, pons and midbrain. SUVr images for the AD subject showed retention in similar brain regions for both tau tracers with the same rank order of regional SUV/SUVr. Notably, for this subject, [18F]APN-1701 continued to accumulate up to the end of image acquisition.

Conclusions: [18F]APN-1701 demonstrates cortical uptake generally similar to [18F]APN-1607 but its slow kinetics are unfavorable for further development.
A VISUAL INTERPRETATION ALGORITHM FOR ASSESSING BRAIN TAUOPATHY WITH 18-F MK 6240 POSITRON EMISSION TOMOGRAPHY

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Aims: In vivo characterization of pathologic deposition of tau protein in the human brain by PET imaging is a promising tool in drug development trials of Alzheimer’s disease (AD). [18F]MK-6240 is a radiotracer with high selectivity and sub-nanomolar affinity for neurofibrillary tangles. Here, we developed a visual assessment that provides a binary outcome for tau deposition, as well as a semi-quantitative metric of the extent of regional tau deposition in the brain.

Methods: Data included 214 subjects (AD, HC, MCI, and other disorders) who underwent [18F]MK-6240 PET scans, which were used to generate 70-90min static images. Half of the subjects were reviewed by an expert nuclear medicine physician blind to participants’ diagnosis to identify common patterns of brain uptake. Based on this review, a visual read method was developed for testing in a separate cohort of 102 subjects, which included two additional independent readers. Visual read outcomes were compared with quantitative ROI analysis.

Results: Readers demonstrated a high level of concordance for binary regional outcome, with all three-reader in agreement on 74.3% of scans with a Fleiss’s k=0.912. Readers showed excellent intra-reader reproducibility, with two readers attaining 10/10 self-agreement and one reader attaining 9/10 agreement. Relative to clinical diagnosis, readers showed a sensitivity of 76-81% and specificity of 91-92%. Mean cortical SUVr was higher in read positive (2.17/0.91) than to negative (1.02/0.18) scans.

Conclusions: We developed a read algorithm permitting both binary determination and the regional extent of abnormal tau. These cross-sectional results demonstrate feasibility, which might allow for further evaluation of progressive tau changes in AD.
COMPARING PLASMA P-TAU181 WITH TAU-PET FOR ALZHEIMER’S DISEASE DIAGNOSIS AND PROGNOSIS

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Aims: To compare plasma pTau181 with tau-PET in a cohort of subjective cognitive decline (SCD), mild cognitive impairment (MCI) and Alzheimer’s disease (AD) dementia participants in their accuracies in discriminating between diagnoses and Aβ-status, and their association with longitudinal cognition.

Methods: One-hundred-and-ten participants from the Amsterdam Dementia Cohort (n=31 Aβ-PET-negative SCD; n=19 Aβ-PET-positive SCD; n=60 Aβ-positive MCI/AD) underwent dynamic [¹⁸F]flortaucipir (tau)-PET and had plasma collected within 12-months from tau-PET. Plasma pTau181 was measured using the Simoa assay (Quanterix). We extracted entorhinal, temporal and neocortical tau-PET binding potential (BPND). We compared the accuracies of pTau181 with tau-PET for discriminating diagnoses (SCD vs. MCI/AD) and Aβ-status (positive vs. negative) using area-under-the-receiver-operating-curve (AUC-ROC) analyses. We compared pTau181 with tau-PET in the association with longitudinal MMSE (spanning 3.2±2.7 years) using linear mixed models (age-, sex- and education-corrected).

Results: For discriminating SCD (regardless of Aβ-status) from MCI/AD, the AUC for pTau181 (0.742[0.646-0.839]) was lower than for tau-PET (entorhinal: 0.889[0.823-0.955]; temporal: 0.922[0.867-0.976]; neocortical: 0.888[0.824-0.952]; all p<0.01) (Fig1A). For discriminating Aβ-negative from Aβ-positive participants (regardless of diagnosis), the AUC for pTau181 (0.877[0.810-0.943]) was lower than for entorhinal BPND (0.950[0.913-0.987], p=0.04), but similar to temporal (0.951[0.910-0.991]) and neocortical BPND (0.862[0.795-0.929]) (both p>0.05)(Fig1B). Within SCD, both pTau181 and tau-PET BPND were associated with decline on the MMSE (beta’s and significance-levels: Fig2). Within MCI/AD, only tau-PET BPND was associated with decline on the MMSE.

Conclusions: Plasma pTau181 is a promising biomarker for identifying Aβ-pathology and selecting subjects at-risk for early cognitive decline. However, for diagnostic and prognostic use in clinical AD, tau-PET is preferred.
**Figure 1** Area under the curve (AUC) analyses for the accuracies of plasma pTau181 and tau-PET in discriminating between SCD and MCI/AD (A), and between Aβ-negative and Aβ-positive participants (B).

![Graph A: pTau181 vs. tau-PET: diagnosis](image)

![Graph B: pTau181 vs. tau-PET: Aβ status](image)

<table>
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<th>MMSE score</th>
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<th>MCI/AD</th>
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</table>

Table 1: Associations of tau-PET and plasma pTau181 with longitudinal MMSE score

Shown are beta estimates and corresponding significance levels from linear mixed models adjusted for age, sex and education (according to Verhage system) to assess associations between tau-PET or plasma pTau181 (predictor variables) and longitudinal MMSE score (outcome variable). Tau-PET, plasma pTau181 and MMSE scores were scaled to enable comparison of effect sizes.

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$
INVESTIGATING THE EFFECT OF TAU DEPOSITION AND APOE ON HIPPOCAMPAL MORPHOMETRY IN ALZHEIMER’S DISEASE: A FEDERATED CHOW TEST MODEL

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Aims: Tau tangle is the specific protein pathological hallmark of Alzheimer’s disease and plays a crucial role in leading to dementia-related structural deformations observed in MRI scans. The volume loss of hippocampus is mainly related to the development of AD. Besides, APOE also has significant effects on the risk of developing AD. However, few studies focus on integrating genotypes, MRI, and tau deposition to infer multimodal relationships. In this paper, we proposed a federated chow test model to study the synergistic effects of APOE and tau on hippocampal morphometry.

Methods: As illustrated in Fig. 1, the image-tau relationship (correlation) is diluted when the population is mixed, but when we stratify the population based on their genotypes, we can observe strong correlations (AA and BB) across subgroups. Therefore, the samples are first stratified into three cohorts according to their APOE genotypes and each imaging biomarker is used as the predictor, and the tau measure (Braak12/Braak34) is used as the response in each group. Then, a p-value is computed with Chow test to evaluate the difference in these cohorts.

Results: We first adopt the hippocampal volume as the imaging biomarker in our model and p-values are significant for both sides hippocampus. (Fig.2 shows Pearson correlation of each group). Then, we apply two morphometry features, radial distance and surface tensor-based morphometry, as the imaging biomarker to figure out the regions where the atrophy focuses (Fig. 3).
Conclusions: In the future, we will use this model to study Amyloid burden and AD-related SNP, like rs11136000 on CLU.
Aims: The complex pathology of AD, newly available large data sources, and advances in computation have spurred the use of data-driven techniques that permit a joint analysis of multiple sources of data. This study examines the statistical reliability of results derived from data-driven decomposition techniques and seeks to formulate hypothesis-driven disease group comparisons from fused features.

Methods: Structural and functional neuroimaging data are paired with single nucleotide polymorphisms (SNP) from 135 patients who are cognitively normal (CN), mild cognitive impaired (MCI) or Alzheimer’s disease (AD) diagnosis. Multi-modal decomposition methods are applied to all possible combinations of FDG-PET, structural MRI (sMRI), and SNP data. The results are grouped using agglomerative clustering, and the centroids are extracted. The cluster compactness index ($I_c$) per component are derived as a measure of cluster tightness, with indices closer to 1 indicating higher statistical reliability.

Results: Single modalities showed poorer compactness than two-way and three-way fusion, with the SNP data showing low statistical reliability ranging between (0.35, 0.46). Improvement was observed for two-way analyses involving SNP information with FDG-PET and sMRI modalities. The highest amount of statistical reliability was achieved for the three-way decomposition, (0.94, 0.99). Spatial maps were identified using the IC centroids and highlighted regions of the brain known to be affected by brain atrophy. T-tests were run on components.

Conclusions: Combining three-way modalities achieved higher statistical reliability and identified significant differences of the MCI vs AD disease statuses using data driven methods then single or pairwise data fusions. This showcases the ability of decomposition methods to permit hypothesis-driven questions when multiple modalities are used.
PROTEOMIC PROFILING OF EXTRANECULAR VESICLES SEPARATED FROM BRAIN AND PLASMA OF CHRONIC TRAUMATIC ENCEPHALOPATHY AND RISK GROUPS

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Aims: Chronic Traumatic Encephalopathy (CTE) is a tauopathy that affects individuals with a history of mild repetitive brain injury. Extracellular vesicles (EVs) carry pathogenic proteins such as aggregated tau and may contribute to the progression of CTE. We aimed to determine the uniquely enriched proteins in plasma EV samples from former National Football (NFL) player with a risk for CTE(CTEr) and brain-derived EVs isolated from CTE and age-matched control cases.

Methods: EVs were separated from former NFL player (8 brain / 30 plasma) and age matched control group (10 brain/25 plasma) by discontinuous sucrose gradient ultracentrifugation or size exclusion chromatography respectively and analyzed by high-resolution liquid chromatography-mass spectroscopy and SIMOA for total or phosphorylated tau(p-tau) measurement.

Results: Level of pT181 tau were significantly upregulated in CTE or CTEr group compared to control in both brain and plasma EVs. Bioinformatic protein-protein interaction analysis of brain-derived EVs revealed the functional interaction of SNAP-25 and PLXNA4 with tau, and a combination of pT181 tau with SNAP-25 or PLXNA4 were able to distinguish two groups by 96.3, and 93.8 % accuracy respectively. A combination of collagen type VI alpha 3 and 1 chain (COL6A3 and COL6A1) and reelin (RELN) in plasma-derived EVs was able to distinguish two groups with 85% accuracy (AUC = 0.85) by machine learning approach.

Conclusions: The combination of PLXNA4 or SNAP-25 and p-tau in cerebrospinal fluid EV may be used to monitor the progression of CTE. COL6A3, RELN and COL6A1 in plasma EVs may serve as the potential diagnostic plasma biomarkers for CTE risk.
Aims: Frontotemporal lobar degeneration (FTLD) is an umbrella term for a group of neurodegenerative diseases affecting the frontal and/or temporal lobes of the brain. As the diagnosis of FTLD relies primarily on a neuropsychological assessment and clinical signs could be shared with primary psychiatric disorders or Alzheimer’s disease frequent misdiagnosis have been reported. TAR DNA binding Protein of 43 kDa (TDP 43) was measured in cerebrospinal fluid as a specific biomarker for certain forms of FTLD. Unfortunately, studies suggest that TDP 43 alone will not be efficient enough in clinical practice. Therefore, in this study we evaluate the ability of the CSF TDP 43 /non-pTau ratio to discriminate FTLD, AD and PPD patients.

Methods: TDP 43 and non-pTau have been measured by ELISA in the CSF of patients with FLTD, Alzheimer’s disease (AD), Lewy Body Dementia (LBD), Parkinson’s disease dementia (PDD) and psychiatric patients as controls.

Results: The data obtained suggest that a ratio between TDP 43 and non-pTau values measured with our ELISA kits in CSF of patients could be useful to support the differential diagnosis of FLTD in association with a complete neuropsychological assessment and brain imaging.

Conclusions: Further studies will show if a ratio between TDP 43 and non-pTau values is suitable for supporting of differential diagnosis of FLTD.
POSTERS

SECERNIN-1 AS NOVEL BIOMARKER CANDIDATE OF ALZHEIMER’S DISEASE

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Aims: Secernin-1 (SCRN1) is a cytosolic 50-kDa protein that is highly expressed in neurons but also other cell types. It has been identified as a component of amyloid plaques and found to interact with phospho-tau aggregates in specific Alzheimer’s disease (AD)-related tauopathies. The objective of this study was to evaluate the potential of cerebrospinal fluid (CSF) SCRN1 as a biomarker of tauopathy in Alzheimer’s disease.

Methods: We developed a mass-spectrometric parallel reaction monitoring (PRM) assay that allowed measurement of SCRN1 in CSF and applied it in a pilot study of patients with AD (n=25) and non-AD (n=36) core biomarker profiles (measured by Lumipulse). In brief, CSF samples were spiked with a stable isotope labelled SCRN1 tryptic peptide for relative quantification and subsequently digested with trypsin. Samples were then analysed by liquid chromatography coupled to a high-resolution orbitrap mass-spectrometer.

Results: CSF SCRN1 concentration was significantly increased in AD compared with controls (p<0.01). It showed moderate positive correlations with total-tau (Pearson r = 0.47, p<0.001) and phospho-tau (Pearson r = 0.47, p<0.001) concentrations, and no correlation with amyloid-β 1-42.

Conclusions: Our results indicate that SCRN1 in CSF is a potential biomarker for AD pathology.
POSTERS

THE PREDICTION OF TAU PATHOLOGY BY VISUOSPATIAL MEMORY IMPAIRMENT AND ENTORHINAL CORTEX ATROPHY IN ALZHEIMER'S CONTINUUM

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Aims: We investigated the association between phosphorylated tau (p-Tau) level and cognitive impairment across the AD continuum and the mediating role of medial temporal lobe (MTL) atrophy. We also developed a prediction model for abnormal tau accumulation.

Methods: We included participants from the Gwangju Alzheimer's Disease and Related Dementia Cohort in Korea, who completed cerebrospinal fluid analysis and clinical evaluation, and corresponded to one of three groups according to the biomarkers of A and T profiles based on the National Institute on Aging and Alzheimer's Association research framework. Multiple linear and logistic regression analyses were performed to examine the association between p-Tau and cognition and to develop prediction models. Receiver operating characteristic curve analysis were performed to examine the discrimination ability of the models.

Results: Among 185 participants, 93 were classified as A-T-, 23 as A+T-, and 69 as A+T+. There was an association between decreased visuospatial delayed memory performance and p-Tau level, independent of other relevant variables (e.g., Aβ). MTL neurodegeneration was found to mediate the association between the two. Prediction models with visuospatial delayed memory alone and visuospatial delayed memory and entorhinal thickness for tau pathology were suggested and they were validated in an independent sample.

Conclusions: Preliminary findings from the current study suggest that combination of visuospatial memory impairment and entorhinal cortex atrophy can predict abnormal tau accumulation in the AD continuum. Suggested models are potentially useful in predicting tau pathology, and might be utilized practically in the field.
Aims: Individuals with high tau accumulation rates are the focus of clinical tau-targeting trials. The polygenic hazard score (PHS) predicts the onset of AD symptoms and is associated with elevated CSF tau pathology. This study aims to investigate whether PHS, which is cheap and quick to determine, could predict which individuals are likely to have higher tau accumulation rates, and thus the most valuable for clinical trials.

Methods: Cross-sectional (N = 142 CN; N = 95 MCI) and Longitudinal (N = 112 CN and MCI) [18F]Flortaucipir data were downloaded from ADNI. The SUVRs of ROIs were normalized by inferior cerebellar GM. The ROIs were the entorhinal cortex and meta-temporal composite regions. The PHS was calculated by incorporating AD-associated SNPs into a survival analysis framework. We used 65th percentile of PHS for stratification: above 65th as high-risk and below 65th as low-risk.

Results: High-risk group had significantly higher entorhinal tau deposition than the low-risk group (CN: p = 0.016; MCI: p = 0.002; covariates: age, sex, education, and amyloid status). For longitudinal analysis, using linear mixed models, the tau accumulation rate in the meta temporal regions was higher in PHS high-risk group in the combined CN and MCI (interaction p = 0.004; covariates: baseline age, sex, education, baseline amyloid status and baseline diagnosis).

Conclusions: Our findings suggest that independent of amyloid status, PHS could be used to cheaply and effectively identify the most appropriate participants for clinical trials where tau accumulation is either a target, an outcome, or both and thus improve the treatment efficacy.
Aims: Frontotemporal dementia (FTD) is a heterogeneous group of disorders of behavior, personality or speech, accompanied by focal degeneration of the frontal and/or temporal lobes. Currently, there are six clinical subtypes of FTD, one of which is FTD with corticobasal syndrome.

Methods: Case report of “possible” FTD with cortico-basal variant.

Results: Patient G., 60, male, 2 years ago was hospitalized with diagnosis “acute cerebrovascular event” due to speech disorders, difficulties in choosing words. Gradually slight decrease in memory for current events, slowness progressed, speech disorders intensified. In neurological status: elements of motor aphasia with agrammatism, perseverations and literal paraphasias. All types of apraxia were revealed: kinetic (in Luria “fist-rib-palm”) with perseverations; kinesthetic apraxia in “finger pose praxis” test; spatial and eye closing apraxia. “alien arm” syndrome, more pronounced on the left, with phenomena of levitation and intermanual conflict. MOCA test - 14 points. MRI: atrophy of the frontal and temporal lobes prevails, predominantly on the right. The patient was diagnosed with “Possible” FTD, cortico-basal variant, in the form of “frontal” syndrome with efferent motor aphasia, visual-spatial agnosia and dysgraphy, “frontal” signs (positive “palm-mouth” and “grasping” reflexes); “cortico-basal syndrome” with gross dynamic, optical-kinesthetic dyspraxia, dermolexia, apraxia of eye closure, “alien arm” syndrome with phenomena of levitation and intermanual conflict, with MRI-verified asymmetric atrophy of the frontal-temporal regions of the brain.

Conclusions: Difficulties in interpreting individual clinical symptoms and their combinations, the diagnosis of FTD symptoms remains a difficult task. Clinical recognition and diagnosis of FTD variants are important in determining prevalence, family counseling and prognosis.
Aims: The immuno-infrared-sensor enables the diagnosis of Alzheimer’s disease by measuring the secondary structure distribution of Amyloid-beta (Aβ) in CSF and blood plasma [1-4]. For this immunoassay an antibody is essential, which binds to all conformations of the biomarker peptide. We developed different immunization strategies to generate monoclonal Aβ-antibodies (mAb) using wt and APP Knock-Out (KO) mice.

Methods: KO and wt mice were immunized with different Aβ peptides. With an antigen-specific ELISA antibody-producing hybridoma cells were screened and selected for performance tests with the IR-sensor. Immuno-infrared-analyses provided a detailed characterization of the structural selectivity of the antibodies.

Results: Both KO and wt mice generated multiple Aβ specific antibodies, though KO mice yielded 8 times more Aβ-positive antibodies than wt. All antibodies, probably polyclonal, revealed binding to alpha-helical or disordered monomeric Aβ as well as to β-sheet enriched Aβ (oligomers, fibrils). Additionally, seven of these antibodies differentiated between AD patients and healthy controls in cerebrospinal fluid (CSF) samples.

RARE MISSENSE VARIANT R251G ON APOE COUNTERBALANCES THE ALZHEIMER’S DISEASE RISK ASSOCIATED WITH APOE E4

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Aims: In addition to rs7412 (ε2) and rs429358 (ε4) missense variants on APOE, we aimed to estimate the Alzheimer’s disease (AD) risk associated with other APOE missense variants.

Methods: As a discovery sample, we queried the ADSP whole-exome and whole-genome sequencing data. As a replication sample, we imputed microarray data from the ADGC on the TOPMed reference panel (keeping cohorts with $r^2 > 0.75$ for considered variants). We kept one copy of duplicated individuals across the discovery and replication sets. To avoid population stratification, we restricted our analysis to European ancestry individuals, and we accounted for remaining population structure and relatedness using the PCAir and PCRelate methods. To disentangle the effect of the common ε2 and ε4 alleles we performed stratified analysis within each APOE main genotype. In primary analyses, we tested the association with AD status using linear-mixed models, and in secondary analyses the association with age-at-onset in AD cases using linear-mixed models and the risk of conversion to AD using competing risk regression.

Results: We identified 3 missense variants with minor allele count above 10 in the discovery sample (Table 1). In ε3/ε4 individuals, R251G was associated with decreased AD risk (OR=0.17, p=4.4e-6, Table 2), a 6-year delayed AD-onset (β=6.04, p=0.08, Table 3) and slower cumulative incidence (HR=0.26, p=2.9e-4, Table 4).

Table 1. Number of carriers in ADSP WES and WGS, among European or Admixed-European groups as determined by SNPWeight. rs769452_C (Leu28Pro) and rs267606661_G (Arg251Gly) are in-phase with APOE-ε4, while rs199768005_A (Val236Glu) is in phase with APOE-ε3.

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Table 2. V236G and R251G are significantly associated with a decreased AD risk in the discovery sample. R251G association significantly replicates in an independent sample.

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<td>7335</td>
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<td>0.17 [0.06; 0.48]</td>
<td>7.8e-4</td>
</tr>
<tr>
<td></td>
<td>p.Arg251Gly</td>
<td>rs267606661</td>
<td>ε4/ε4</td>
<td>924</td>
<td>7</td>
<td>0.35 [0.04; 2.84]</td>
<td>0.33</td>
</tr>
<tr>
<td>Replication</td>
<td>p.Val236Glu</td>
<td>rs199768005</td>
<td>ε3/ε3</td>
<td>5741</td>
<td>10</td>
<td>0.40 [0.10; 1.59]</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>p.Arg251Gly</td>
<td>rs267606661</td>
<td>ε3/ε4</td>
<td>4630</td>
<td>16</td>
<td>0.19 [0.07; 0.54]</td>
<td>1.7e-3</td>
</tr>
<tr>
<td>Meta-analysis</td>
<td>p.Val236Glu</td>
<td>rs199768005</td>
<td>ε3/ε3</td>
<td>18345</td>
<td>27</td>
<td>0.34 [0.15; 0.75]</td>
<td>7.3e-3</td>
</tr>
<tr>
<td></td>
<td>p.Arg251Gly</td>
<td>rs267606661</td>
<td>ε3/ε4</td>
<td>11965</td>
<td>34</td>
<td>0.18 [0.09; 0.37]</td>
<td>4.4e-6</td>
</tr>
</tbody>
</table>

Table 3. R251G is associated with a delayed age-at-onset in ε3/ε4 carriers. Linear mixed-models association with age-at-onset in AD cases.

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>MAC</th>
<th>Beta [95% CI]</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discovery</td>
<td>4318</td>
<td>4</td>
<td>3.94 [-4.31; 12.2]</td>
<td>0.35</td>
</tr>
<tr>
<td>Replication</td>
<td>2511</td>
<td>2</td>
<td>10.27 [-1.45; 21.99]</td>
<td>0.09</td>
</tr>
<tr>
<td>Meta-analysis</td>
<td>6829</td>
<td>6</td>
<td>6.04 [-0.71; 12.79]</td>
<td>0.08</td>
</tr>
</tbody>
</table>
Table 4. R251G is associated with a less pronounced cumulative incidence of AD in ε3/ε4 carriers. Competing risk regression accounting for the competing risk of death, and for right censoring at last exam for controls.

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>MAC</th>
<th>HR [95% CI]</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discovery</td>
<td>6731</td>
<td>17</td>
<td>0.27 [0.1; 0.69]</td>
<td>6.6e-3</td>
</tr>
<tr>
<td>Replication</td>
<td>4040</td>
<td>13</td>
<td>0.26 [0.09; 0.78]</td>
<td>0.02</td>
</tr>
<tr>
<td>Meta-analysis</td>
<td>10771</td>
<td>30</td>
<td>0.26 [0.13; 0.54]</td>
<td>2.9e-4</td>
</tr>
</tbody>
</table>

Conclusions: R251G counterbalances the risk associated with APOE-ε4. Additional studies elucidating the biochemical properties behind this protective effect could lead to novel approaches to mediating the APOE-ε4 risk.
ASSOCIATION OF MAPT H1/H2 HAPLOTYPES WITH BASELINE TAU BURDEN AS MEASURED BY PLASMA TAU AND FLORTAUCIPIR PET

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1Eli Lilly & Company, Cambridge Innovation Center Neuroscience, Cambridge, United States of America, 2Avid Radiopharmaceuticals, Neuroscience, Philadelphia, United States of America, 3Eli Lilly and Company, Statistics, Indianapolis, United States of America

Aims: We report association analysis of the MAPT gene H1/H2-haplotype and tau burden, as measured by plasma pTau and Flortaucipir-PET. The H1-haplotype is associated with increased risk of developing certain tauopathies, however, the risk conferring effect of H1/H2-haplotypes in AD remains unclear.

Methods:

Patients from past trials conducted by Eli Lilly used in this study

<table>
<thead>
<tr>
<th>Clinical Trial (ClinicalTrials.gov ID)</th>
<th>n (pTau181,pTau217)</th>
<th>n (Flortaucipir-PET)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXPEDITION-1 (NCT00905372)</td>
<td>282,281</td>
<td>--</td>
</tr>
<tr>
<td>EXPEDITION-2 (NCT00904683)</td>
<td>320,322</td>
<td>--</td>
</tr>
<tr>
<td>EXPEDITION-3 (NCT01900665)</td>
<td>435,137</td>
<td>182</td>
</tr>
<tr>
<td>NAVIGATE-AD (NCT02791191)</td>
<td>--</td>
<td>223</td>
</tr>
</tbody>
</table>

Single nucleotide polymorphism rs1800547 was used to tag H1/H2-haplotypes in MAPT based on prior literature showing strong linkage disequilibrium

Results: We used multiple linear regression to predict log-transformed, normalized baseline pTau181 or pTau217 from H2-Haplotype status controlling for age, sex, and APOE4 in EXPEDITION-1/2/3. The models showed statistically significant increase in pTau associated with the presence of H2-haplotype. Using ANCOVA, we confirmed higher Tau burden in H2-haplotype using flortaucipir uptake differences in temporal lobe controlling for age and sex in EXPEDITION-3 and NAVIGATE-AD.

Conclusions: Our analyses suggest higher Tau burden in H2-haplotype carriers, contrasting with prior publications reporting reduced risk for H2 carriers in neurodegenerative diseases. Further analysis in larger well-characterized cohorts is needed to validate our findings and to provide conclusive evidence on the risk or protective effect conferred by H2-haplotype in AD.
Aims: Existing metabolite quantitative trait loci (mQTL) were found using tissues other than brain, which being less ideal for studying neurodegenerative diseases, such as AD. We aim to discover novel associations between metabolites and genetic loci using two brain metabolomics datates, Knight ADRC together with the Dominantly Inherited Alzheimer Network (DIAN), and the Religious Orders Study and Memory and Aging Project (ROSMAP). In addition, we aim to discover novel genetic-metabolite associations in the central nervous system (CNS) combining a recently published mQTL study using cereal spinal fluid (CSF). Moreover, we aim to find causal metabolites to brain disorder such as AD and PD.

Methods: The datasets were generated by Metabolon platform. The Knight ADRC and DIAN dataset was generated by our lab, and the ROSMAP dataset was download from Synapse (syn26007830). We firstly performed association study on two brain datasets separately using linear regression model adjusted for age at death, sex, genetic components, and genotype array methods. We secondly performed meta-analysis for the 539 shared metabolites between two brain studies using METAL inverse-variance weighted fixed approach. We will be performing meta-analysis for the two brain studies and the CSF study using METASOFT RE2 random model.

Results: We performed multiple test correction using the number of principle components that explains 95% variance of analyzed metabolites. The meta-analysis of brain studies generated 18 mQTLs, in which six associations are novel based on the SNiPA database (v3.4).

Conclusions: In conclusion, our analysis discovered novel metabolite quantitative traits, and could be beneficial to study neurological disorders.
CONSEQUENCES OF A MATERNAL HIGH FAT DIET DURING LACTATION ON MALE AND FEMALE OFFSPRING IN A MOUSE MODEL OF TAUOPATHY

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¹Université de Lille, Inserm, CHU Lille, UMR-S1172, Lilncog - Lille Neuroscience & Cognition, Lille, France, ²Université de Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, Us 41-ums 2014 – Plbs, Animal Facility, Lille, France, ³Université de Lille, Inserm, CHU Lille, Institut Pasteur de Lille, U1011 Egid, Lille, France, ⁴Université de Lille, Inserm, U1192 – Laboratoire Protéomique, Réponse Inflammatoire Et Spectrométrie De Masse (prism), Lille, France, ⁵CNRS UMR 7104, Inserm, Université de Strasbourg, U1258, Genomeast Platform, Institut De Génétique Et De Biologie Moléculaire Et Cellulaire (igbmc), Lille, France

Aims: Accumulating data support the role of environmental factors in the modulation of tau pathology and related cognitive decline in Alzheimer’s disease, but the putative influence of perinatal environment on these mechanisms remains contradictory and under-investigated. Here, we evaluated the effects of a maternal high-fat diet during lactation on the development of tauopathy in the THY-Tau22 strain, a model of progressive tau pathology and cognitive decline.

Methods: During lactation, mothers receive either a chow (13.6% of fat) or high-fat diet (mHFD, 58% of fat). At weaning, offspring were placed under a control diet (8.4% of fat) until sacrifice at 4 months (i.e. beginning of tau pathology), or at 7 months of age (i.e. beginning of memory impairment).

Results: In males, mHFD increased hippocampal tau pathology at 4 months of age and led to an impairment of short-term spatial memory in 7-months-old animals. In females, mHFD increased tau pathology at 7 months of age. Our data therefore indicate the existence of a sexual dimorphic effect of mHFD in the THY-Tau22 model with the male offspring being impacted earlier. Using RNA-seq, we performed a time-course analysis of hippocampal transcriptome changes between 4- and 7-months-old. Our results indicate that mHFD strengthens age-related development of transcriptomic changes in THY-Tau22 male mice, with deregulated genes associated to extracellular matrix, cell adhesion, mitochondria and synapse.

Conclusions: Together, our data showed for the first time that mHFD has long-lasting and sex-specific effects on the development of tauopathy and reinforced the idea that age-associated neurodegenerative diseases may have neurodevelopmental origin.
Aims: in tropical settings, confirmation of the etiology of atypical and secondary parkinsonian syndrome is difficult to establish because of undermedicalization. We report 73 of cases of atypical parkinsonian syndrome diagnosed clinically on the United Kingdom Parkinson’s disease Brain Bank (UKPDSBB) criteria for diagnosis of parkinsonism, imaging and biological data.

Methods: This was a prospective study of descriptive type with a duration of 5 years from 04 January 2016 to 05 January 2021 carried out in the neurology department of the University Hospital of Conakry. Were included in the study all patients with an etiological diagnosis of an atypical Parkinsonian based on the criteria of the UKPDSBB for the diagnosis of Parkinson's and the imaging and biological data.

Results: Two (2) rare etiologies were identified: HIV infections with 8 cases, opportunistic infections: 12 cases, neurosyphilis: 2 cases, vascular causes: 6 cases and 15 cases identified in areas with bauxite production in an environment of probable intoxication. The correlations between these parkinsonian syndromes and alumina intoxication are discussed. Parkinsonian syndrome of iatrogenic cause: neuroleptics, antidepressants, anti-epileptics: 13 cases, hereditary parkinsonian syndromes: 8 cases, Wilson's disease: 3 cases and other syndromes: 6 cases.

Conclusions: Parkinsonian syndromes are under-reported in Guinea and the causes are of infectious origin: intoxications, notably by alumina, and iatrogenic.
INVESTIGATING ISOFORM-SPECIFIC FUNCTIONS OF TAU UNDER HEALTHY AND PATHOLOGICAL CONDITIONS USING TAU-DEFICIENT HIPSC-DERIVED CORTICAL NEURONS

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University Hospital Cologne, Institute Of Human Genetics, Cologne, Germany

**Aims:** One hallmark of many neurodegenerative diseases, such as Alzheimer’s disease (AD), is the formation of neurofibrillary tangles by hyperphosphorylated Tau in the brain, resulting in neuronal death and cognitive decline. In the adult human brain, six Tau isoforms are expressed, originating from alternative splicing of exons 2, 3, and 10 of the MAPT gene. The isoforms differ in the number of N-terminal inserts (0, 1, or 2N) and the C-terminal repeat number (3 or 4R). Recent results from murine neurons highlight that the six human-specific isoforms are differentially localized within neurons and influence microtubule dynamics in an isoform-specific manner. In this study, we aim to generate a human neuronal model system to further characterize the human-specific Tau isoforms under basic and pathological conditions.

**Methods:** We successfully generated three Tau KO hiPSC cell lines derived from Ngn2-WTC11 cells using CRISPR/Cas9 and characterized the generated cell lines using genetic, biochemical and microscopy-based methods.

**Results:** No difference between Tau KO and WT hiPSCs was observed in the capability of neuronal differentiation or neuronal morphology. Initial results from re-expressed Tau isoforms in Tau KO human neurons support the differential localization of the isoforms observed previously. Further experiments will investigate the isoform-specific role of Tau regarding microtubule dynamics, axonal branching, and synapse formation under basal and pathological conditions.

**Conclusions:** Tau KO hiPSCs are a versatile tool to study basic as well as pathological functions of Tau. Expected results will provide a major understanding of Tau function and help identifying potential therapeutic targets for the treatment of AD and related neurodegenerative diseases.
ELUCIDATING THE ROLE OF TAU ISOFORM EXPRESSION IN HUMAN IPSC-DERIVED TAUOPATHY MODELS

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¹Ludwig-Maximilians-Universität München, Institute For Stroke And Dementia Research, University Hospital Munich, Munich, Germany, ²Ludwig-Maximilians-Universität, Graduate School Of Systemic Neurosciences, Planegg-Martinsried, Germany, ³SyNergy, Munich Cluster For Systems Neurology, München, Germany

Aims: Malfunction of Tau is a hallmark of neurodegenerative Tauopathies such as Alzheimer’s Disease and Frontotemporal Dementia (FTD) with Tau mutations causing familial FTD. Tau expression and splicing are highly regulated and mis-regulation of the two classes of splice isoforms, 3R and 4R Tau, leads to FTD. Despite its importance in physiology and disease, currently available Tauopathy models largely do not recapitulate adult human Tau isoform expression at a 3R to 4R ratio of 1:1.

Methods: We developed a CRISPR/Cas9 genome editing strategy to alter Tau isoform expression from the endogenous MAPT locus in human induced pluripotent stem cell (iPSC)-derived cortical neurons. In addition, we included pathogenic Tau mutations to investigate disease phenotypes. We culture these neurons in 2D as well as in 3D culture.

Results: We observed that our edited neurons indeed express a 1:1 ratio of 3R and 4R Tau isoforms much earlier than in unedited wild type neurons. Currently, we investigate the effect of pathogenic Tau mutations in a background of adult human 3R:4R Tau isoform expression to investigate differences in formation of disease phenotypes.

Conclusions: Using CRISPR/Cas9 genome editing, we can force expression of 4R Tau already in young iPSC-derived neurons. Our model increases the utility of iPSC-derived neurons to study adult-onset neurodegenerative Tauopathies and provides a novel platform to investigate 3R/4R splice ratio-mediated differences in Tau biology and pathology.
ENDOGENOUS 3R:4R TAU RATIO IN MICRORNA-MEDIATED DIRECTLY REPROGRAMMED HUMAN NEURONS

L. Capano¹, E. Ficulle², C. Sato³, K. Horie³, R. Bateman³, C. Karch⁴, K. Duff⁵, A. Yoo¹
¹Washington University in Saint Louis, Developmental Biology, Saint Louis, United States of America, ²University College London, Uk Dementia Research Institute, London, United Kingdom, ³Washington University in St Louis, Neurology, Saint Louis, United States of America, ⁴Washington University in St Louis, Psychiatry, Saint Louis, United States of America, ⁵University College London, Dementia Research Institute, London, United Kingdom

Aims: Current tauopathy human cell models cannot recapitulate endogenous expression of the six developmentally regulated adult tau isoforms, specifically 3R and 4R families defined by the exclusion or inclusion of exon 10, respectively. Human adult brain expresses 3R and 4R at a 1:1 ratio and a ratio imbalance is sufficient to drive tauopathy. We asked if direct reprogramming of human fibroblasts into neurons, bypassing the stem cell stages, would recapitulate adult isoforms of tau.

Methods: MicroRNAs (miRNAs) miR-9/9* and miR-124 function as potent reprogramming effectors to direct reprogramming of adult human fibroblasts to neurons (miNs) with high efficiency. Reprogrammed neurons were analyzed for 3R and 4R tau expression at the transcript and protein levels.

Results: Transcript assays and mass spectrometry demonstrate miNs from adult fibroblasts and adult human brain have indistinguishable 4R profiles and express 3R and 4R tau at a 1:1 ratio, analogous to human adult brain. This starkly contrasts the 4R expression in primary fetal human neurons and iPSC-derived neurons. Endogenous tau isoform regulation in miNs is sensitive to IVS10+16 familial tau mutations and reprogrammed miNs show an increased 4R:3R tau ratio and formation of insoluble tau. Comparing miNs versus iPSC-derived neurons, we identified differentially expressed, age-associated factors which may play a role in exon 10 splicing.

Conclusions: We have defined a novel cellular model for studying all tau isoforms present in the adult human brain through robust miRNA-mediated direct reprogramming. This system can be used as a platform for identifying and testing splicing factor modulators for correction of altered 3R:4R ratio.
DIRECTLY CONVERTED FTD NEURONS SHOW 4R TAU EXPRESSION AND INSOLUBLE AGGREGATES FORMATION

E. Ficulle¹, L. Capano², H. Houlden³, J. Rohrer⁴, A. Yoo², K. Duff¹
¹University College London, U.K Dementia Research Institute, London, United Kingdom, ²Washington University School of Medicine, Department Of Developmental Biology, Saint Louis, United States of America, ³University College London, Department Of Neuromuscular Diseases, UCL Queen Square Institute Of Neurology, London, United Kingdom, ⁴UCL Queen Square Institute of Neurology, Dementia Research Centre, London, United Kingdom

Aims: Tau is a microtubule-binding protein expressed in neurons. In the healthy adult human brain is expressed in 6 different isoforms that can be grouped into 3R and 4R tau present in an equal 1:1 ratio. Tau is the main hallmark of several neurodegenerative diseases, however its role is poorly understood also due to the lack of a reliable in-vitro model that expresses both 3R and 4R isoforms. Using microRNA-direct-conversion, we have developed a human model that recapitulates endogenous tau expression and demonstrates tau pathology.

Methods: Fibroblasts from healthy donors and IVS10+16 FTD-patients were directly converted to age-maintained neurons (miNs) overexpressing miRNAs-9/9*-124. Tau expression and aggregation was characterised via WB, MS, PCR, biosensors, and immunofluorescence.

Results: We have demonstrated the endogenous expression of all 6 tau isoforms in miNs and that the 3R:4R isoform ratio reflects the genotype of the fibroblasts. 10+16 miNs form more insoluble tau aggregates positive for different pathological tau antibodies, including AT-8, TOC1 and MC1. Finally, seeding activity was detected in the 10+16 miNs compared to healthy controls.

Conclusions: We have defined miNs as a model for the endogenous expression of all 6 tau isoforms and demonstrated endogenous aggregation without additional perturbation. This overcomes the limitations of iPSC-neurons which do not physiologically recapitulate the aged human brain and nor form pathological aggregates. Our results demonstrate that we have developed an in-vitro model of tauopathy that reflects adult tau splicing and replicates pathological tau phenotypes. This model can be used to further characterise the pathophysiological role of tau in disease.
Aims: Tau aggregation and its cellular propagation plays a crucial role in the pathology of several neurodegenerative diseases, especially Alzheimer’s disease (AD). The process of propagation is mediated by extracellular tau, which is taken up by cells and serve as seeds for tau aggregation. The development of new compounds to block tau seeding or uptake activity is currently an active field of research. Consequently, reliable in vitro models mirroring this tau pathology are needed.

Methods: To monitor tau seeding and uptake, we established in vitro assays in different cell types: (1) stably transfected tau overexpressing SH-SY5Y cells (2) mouse primary neurons isolated from wild type mice (3) mouse primary neurons isolated from Tau P301S (PS19) mice. Cells were treated with tau seeds isolated from human AD brains (AD-tau seeds) in the presence of lipofectamine to induce tau seeding or in the absence of lipofectamine to monitor tau uptake. As positive control, the anti-tau antibody HT7 was co-incubated with AD-tau seeds, to counteract tau seeding and uptake. Tau aggregation was assessed using the HTRF-based Tau Aggregation Kit from Cisbio.

Results: Tau seeding and uptake was detectable as increased HTRF signal after incubation of all cell types with human AD-tau seeds compared to vehicle control. The HT7 antibody significantly reversed the AD seed associated tau aggregation in the seeding and uptake assay.

Conclusions: The here presented in vitro systems for tau seeding and uptake are suitable to screen for the activity of compounds that block tau propagation.
HOMEOSTATIC SCALING ALTERS SPINE MORPHOLOGY AND PROTEIN COMPARTMENTALISATION OF THE PHOSPHATASE STEP61

D. Taylor¹,², A. Kneysberg¹,², J. Götz¹,²
¹The University of Queensland, Queensland Brain Institute (qbi), Brisbane St Lucia Campus, Australia, ²The University of Queensland, Clem-jones Centre For Ageing Dementia Research, Brisbane, Australia

Aims: Dendritic spines constitute a specialized neuronal compartment that contains the postsynaptic signalling machinery that is essential for synaptic transmission. Spine morphology is a critical determinant of synaptic transmission, affecting the diffusion and compartmentalisation of signalling and scaffolding proteins. One such regulating protein is the striatal enriched phosphatase (STEP61), which dephosphorylates activity-dependent glutamate receptors, targeting them for endocytosis, and dampens synaptic signalling cascades through modulation of kinase activity. However, the global changes in spine morphology have not been investigated together with key regulating synaptic enzymes. Here, we aimed to elucidate the morphology of spines and the localisation of STEP61 on a global scale in a homeostatic scaling paradigm using primary hippocampal neurons.

Methods: We treated wild-type and STEP61 knock-out hippocampal neurons with tetrodotoxin (TTX) or bicuculline (Bic) for 24 h to induce a homeostatic scaling paradigm. Using a fluorescent marker, neuronal spines were visualised and quantitatively analysed with Neurolucida (MBF). More than 15,000 spines were compared per treatment group and genotype to obtain volumetric and protein localization data.

Results: We found that homeostatic scaling bi-directionally altered STEP61 activity, significantly changed spine morphologies and the distribution of spine types across neurons, as well as the distribution of synaptic proteins within spines.

Conclusions: Our findings reveal that synaptic scaling alters global spine morphology and compartmentalisation of key regulating proteins. We also demonstrate alteration of phosphatase activity as the knockout of STEP61 significantly increased spine size but not compartmentalisation and decreased overall spine number.
Aims: Alzheimer's disease (AD) is a neurodegenerative disease and an example of tauopathies with a marked increase in microtubule-associated protein tau aggregation in affected brain regions of dementia patients. Under pathological conditions, tau detaches from the microtubule, accumulates in the cytosol and initiates the fibrillogenesis cascade. In this study, we aim to study the role of end binding proteins in regulating tau attachment or dissociation from microtubule and in AD pathogenesis.

Methods: We knocked down end binding proteins (EBs) in tau-P301L overexpressed SH-SY5Y cells and characterized the extent of tau dissociation from microtubule, tau aggregation and spreading, as well as tau-induced cell death. We also conducted bioinformatics analysis using microarray mRNA and proteomic datasets from human brain tissues of AD patients and healthy controls to determine whether EBs play a role in AD pathogenesis.

Results: We showed that there was more tau dissociation from microtubule, leading to an increase in tau aggregation and cell death in EBs knock-down SH-SY5Y cells. We also illustrated that reduction in EBs leads to an increased tau secretion, suggesting that EBs modulate tau spreading. From our bioinformatics analysis, we identified EBs as differential expressed genes, which were significantly downregulated in AD in both mRNA and protein levels. Importantly, miRNA that has been shown to regulate EBs was significantly altered in AD blood datasets, making it a potential biomarker to correlate with the expression of EBs.

Conclusions: We proposed that EBs play a role in AD pathogenesis and drug compounds targeting EBs might be potential therapeutics.
Aims: Expression datasets, such as those from RNAseq and mass-spec platforms, have been growing at an ever-increasing rate and are relevant to a wide variety of diseases, however, it remains challenging to yield interpretable and actionable results. In this poster, we present novel computational methods that attempt to resolve this issues and demonstrate their use in several Alzheimer's Disease datasets.

Methods: Two publicaly available datasets were used for this analysis: a post-mortem single-nucleus RNAseq dataset from the entorhinal cortex of Alzheimer's Disease and non-demented donors (Leng et al., 2021, Nature Neuroscience) and a post-mortem single-soma RNAseq dataset from the prefrontal cortex of Alzheimer's Disease donors where tangle-bearing neurons were sorted prior to sequencing (Otero-Garcia et al., 2020, bioRxiv). In brief, our method, which we have named GeneFunnel, firstly optimizes filtering steps and statistical correctness (such as pseudobulking) to yield robust gene set enrichment results. Gene set enrichment is then used to guide prioritization of differentially expressed genes using hierarchial clustering and graph theory, focusing especially on the overlap of genes between gene sets.

Results: Using several forms of visualization, we demonstrate how GeneFunnel can create a clear and succint overview of the processes involved in Alzheimer's Disease. As the name implies, the results are made intuitively explorable, effectively facilitating a "funnel" that allows inspection at broad and narrow levels of depth.

Conclusions: We show a novel approach to tackling large and complex expression datasets, demonstrating its utility in summarizing key molecular changes that take place in Alzheimer's Disease.
Aims: Alzheimer's disease (AD), the most common form of dementia, is characterized by the presence of β-amyloid plaques and neurofibrillary tangles (containing hyperphosphorylated Tau protein). Aging is suggested to be the main AD risk factor. Dysregulated epigenetic mechanisms such as histone acetylation have been associated with both aging and AD. However, whether/how aging-deregulated events contribute to AD is unknown. We aimed to establish epigenetic/transcriptomic signatures in physiological and pathological AD/Tau aging.

Methods: We investigated the marker of active transcription H3K27ac at genome-wide level (ChIP-seq) and transcriptomics (RNA-seq) in the hippocampus of 12-month-old THY-Tau22 mice (tauopathy) as well as 18-versus 3-month-old WT mice (aging).

Results: Epigenomic data revealed opposite H3K27ac enrichments between aging and tauopathy: synaptic transmission and glutamate receptor genes were found enriched in tauopathic hippocampi whereas these regions were depleted in aged ones. At transcriptomic level, while aging and tauopathy shared a common inflammatory signature, cholesterol metabolism-genes were found exclusively decreased in tauopathic mice. Excitingly, epigenetic treatment using the histone acetyltransferase activator CSP-TTK21 restored recognition memory in tauopathic mice. Additionally, this treatment rescued the expression of cholesterol metabolism genes and proper epigenomic signatures, suggesting a link between these two events.

Conclusions: Overall, our findings suggest that tauopathy is not an exacerbation of the epigenetic drift observed during the aging process but rather an epigenetic/histone acetylation upsurge resulting from the dysregulation of the cholesterol pathway. Further, CSP-TTK21 revealed to be a promising new “epidrug” to counteract memory impairment, through modulation of brain cholesterol biosynthesis, in the pathological condition of AD.
Aims: This study understands the influence of epigenetic changes particularly DNA methylation, a major modification, in the prefrontal cortex and hippocampus of diabetic brain and its notable effect on the cellular chaperones and synaptic fidelity.

Methods: Chronic high fat diet and STZ-induced diabetic mice were studied for the cognitive dysfunction, and global DNA methylation. Further, its effect on the cellular chaperones and synaptic proteins were examined using DNMT inhibitor, 5-aza-2'-deoxycytidine (5-aza-dC)-via intracerebroventricular injection. Moreover, % methylation of these proteins were also studied, so as to correlate its epigenetic involvement. Computationally, its interaction with the DNMT enzyme were also studied using bioinformatic tools. Histological studies for morphological alterations and neuronal degeneration were also studied..

Results: Altered global DNA methylation and increased levels of DNMTs within the nucleus were confirmed in the cortex and hippocampus of the diseased mice, suggesting hypermethylation at a genetic level. Treatment with AzadC, a global DNA demethylating agent, ameliorated the protein and gene expression of the cellular chaperones and synaptic fidelity. Furthermore, the methylation analysis profile showed, hypermethylation of the hsf1 protein, a master regulator for chaperones and thus, confirmed the epigenetic involvement in the diseased brain. Morphological improvements and decreased neurodegeneration, along with enhanced neurogenesis in the treatment group, suggest that epigenetic modulations do participate in learning and memory. This is supported by the improved behavioral test battery seen in the treatment group.

Conclusions: DNA methylation, could possibly accord in dysregulating the memory-associated proteins at chronic stages in type 2 diabetes.
THE INHIBITION OF LSD1 VIA SEQUESTRATION CONTRIBUTES TO TAU-MEDIATED NEURODEGENERATION


Aims: Alzheimer’s disease (AD) is characterized by the abnormal aggregation of β-amyloid plaques and neurofibrillary tangles of hyperphosphorylated tau (NFTs). However, the molecular mechanism underlying neuronal cell death is not well understood. The lysine-specific histone demethylase, LSD1 functions in nucleus to regulate gene expression by repressing genes. Surprisingly, our lab found that LSD1 is mis-localized with cytoplasmic pathological tau in human AD cases, that suggests LSD1 may be inhibited by tau. To study the function of LSD1, our lab showed that the inducible deletion of LSD1 in adult mice induces cortical and hippocampal neurodegeneration, learning and memory deficits, and transcription alternations that match human AD cases. Therefore, we hypothesized that pathological tau contributes to neuronal cell death by sequestering LSD1 into the cytoplasm.

Methods: To address this hypothesis, we utilized the PS19 tauopathy mouse model to examine the functional consequences of changing LSD1 expression and to examine the interaction between pathological tau and LSD1.

Results: We found that LSD1 is depleted from the nucleus and mis-localized with cytoplasmic NFTs in adult PS19 Tau mice, supporting the hypothesis that NFTs sequester LSD1. In addition, we found that changing LSD1 expression can specifically modulate tau-mediated neurodegeneration in PS19 mice. This suggests that pathological tau is functioning in part through LSD1. Finally, we found that LSD1 interacts with pathological tau through its N-terminus.

Conclusions: We conclude that pathological tau functions in part by sequestering LSD1 in the cytoplasm and inhibiting its function. A model for the pathological interaction between LSD1 and tau will be presented.
OBSERVING NEURODEGENERATION IN REAL TIME USING 2-PHOTON MICROSCOPY IN THE LSD1 INDUCIBLE MOUSE MODEL

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Aims: AD is characterized by two hallmark pathologies: the accumulation of pathological Aβ plaques and neurofibrillary tau tangles (NFTs). However, the role of NFTs in mediating neurodegeneration remains unclear. We have previously demonstrated that the nuclear histone demethylase LSD1/KDM1A is mislocalized to cytoplasmic NFTs in AD cases. In agreement with this, we discovered that hippocampal deletion of LSD1 resulted in widespread loss of pyramidal neurons, a prominent feature of AD. Additionally, the neurodegeneration caused by loss of LSD1 is associated AD specific gene expression changes and learning and memory deficits, suggesting that the neurodegeneration in the LSD1 mouse model may be related to AD patients. In order to better understand neuronal cell death mechanisms, we propose using two-photon live imaging in the LSD1 inducible knockout mouse to visualize neurons dying in the cerebral cortex.

Methods: To visualize neurons dying in the inducible LSD1 mouse model, we incorporated a Thy1-YFP neuronal reporter. Using a two-photon microscope, we will be able to visualize YFP-expressing neurons in an awake animal through a surgically implanted cranial window.

Results: Given the robust neurodegeneration phenotype, loss of LSD1 will generate a feasible and rapid timescale to view neurons degenerating in real time.

Conclusions: Previously, we have shown that axons and dendrites are completely lost from hippocampal neurons in the LSD1 inducible knockout mice. Using live imaging, we hope to determine whether this is an active part of neuronal cell death in this model. In addition, we will interrogate the role of the microglia activation in this system.
TAU PATHOLOGY CAN BE ASSESSED IN WHOLE HEMISPHERES OF THY1 P301S MICE USING LIGHT SHEET MICROSCOPY

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Aims: Intracellular tau inclusions are the neuropathological hallmarks of Alzheimer’s disease (AD). AD tau pathology spreads in the brain through synaptically connected neurons. To demonstrate regional pathology progression in transgenic Tau mouse model, we performed immunolabeling of tau inclusions with phospho-Tau antibody AT8 in Thy1.P301S tau transgenic mice.

Methods: Using light sheet microscopy (LSM), we visualized the 3D distribution of Tau inclusions at cellular resolution in whole hemispheres and its age-dependent increase. To visualize the interconnection of AT8+-neurons, the image stack was reconstructed in 3D using Arivis Vision4D software. Its machine learning algorithm was used to remove unspecific signal originating from blood vessel autofluorescence and tissue surface reflection.

Results: The results of this analysis show a specific detection of the tau inclusions and a greatly improved signal-to-background ratio compared to visualization of the raw signal. Furthermore, this analysis can quantify the occurrence of tau inclusions in terms of number and volume, while decreasing the analysis time by about 70%. To quantify tau aggregates within distinct brain regions, we aligned the datasets to the Allen Brain Atlas. With such data processing, we improved visualization of disease hallmarks in 3D; reduced image analysis time, while increasing throughput; obtained quantitative information on tau inclusions in specific brain areas. Consistent with previous published histological findings, tau inclusions first appear in this animal model in the brain stem and upon ageing progress into the cortex.

Conclusions: Future investigation with LSM will further explore the development of tau pathology, including neuron-to-neuron propagation upon induction by injection of Tau seeds.
A NOVEL KNOCK IN MOUSE MODEL OF ALZHEIMER’S DISEASE

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Aims: Alzheimer’s disease (AD) primary cause of dementia in the elderly, is characterized on a neuropathological level by extracellular accumulation of amyloid peptide and intraneuronal aggregation of Tau proteins found abnormally and hyperphosphorylated. This Tau aggregation causes neuronal death that leads to cognitive impairment. Most of the existing animal models mimic AD pathology by overexpressing both proteins.

Methods: We have generated a new mouse model by crossing a Knock In humanized mouse model for amyloid peptide (APPNL-GF, Saito et al., 2014) with a Knock In mouse model (Tau KIV5) bearing a human mutated 1N4R isoform of Tau (under the control of the murine Mapt promoter) in order to obtain a model that develops concomitant pathologies linked to the co-expression of the two humanized proteins.

Results: The new model was characterized at 6 months on metabolic and behavioral levels but also molecular and biochemical ones. Results revealed absence of any metabolic phenotype (in Chow diet), expression of transgenes and corresponding proteins with increased phosphorylation in the cortex as well as an increasing number of amyloid plaques within the double mutants (APPxTau mice). Increasing neuroinflammation by q-PCR related to amyloid pathology was also detected in the hippocampus.

Conclusions: At this age, neuronal degeneration could not be observed. Further investigation is currently done on 12 month-mice to explore hyperphosphorylation and neurodegeneration using immunohistochemistry as well as behavioral approach to detect any cognitive impairment. This model could serve to investigate aggravating or protective factors of Tau pathology and therefore neurodegeneration.
DIFFERENT TAU DISTRIBUTION PATTERNS IN MAPT MUTANT KNOCK-IN MICE

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Aims: Accumulation of the protein tau is correlated with cognitive impairment in frontotemporal lobar degeneration (FTD-tau), which is characterised by focal atrophy of the temporal lobe. Mutations in the tau gene (MAPT) have been shown to cause FTD but neuroanatomical studies have grouped different mutations together and the heterogeneity of the tau distribution pattern associated with a particular MAPT mutation is poorly understood. Importantly, due to the relative low incidence of FTD little is known about the early neuropathological stages. Here, we generated new mouse models to study the spatio-temporal distribution of tauopathy caused by different MAPT mutations.

Methods: CRISPR/Cas9-based genome editing technology, Base Editor, was used to generate mutant and wild-type humanised MAPT knock-in (hTauKI). Histological and biochemical methods were used to characterise these models.

Results: Three strains were compared: a wild-type control and two lines expressing exonic mutations. Immunohistochemical analysis showed a different spatio-temporal distribution of tau associated with the mutations, whilst biochemistry studies isolated insoluble, seeding tau in only one of the lines. Subsequently, the mutant hTau-KI mice were crossbred with mutant App-KI to accelerate tau pathology in the brain. Whilst one of the APP/tau lines showed pre-tangle pathology, the other displayed sparse but robust AT8, MC1 and Gallyas-positive NFTs in the pyramidal cell layer of the hippocampus and EC. Different tau distribution patterns were observed.

Conclusions: Exonic mutations in MAPT drive tau pathology along different trajectories. These novel, physiologically-relevant tauopathy mouse models will be useful to understand the pathophysiology of tau and the cause of phenotypic diversity in tau-associated diseases.
Aims: Aberrant, chronic neuroinflammation, along with cognitive decline are hallmarks of aging and Alzheimer’s diseases (AD) and related dementias (ADRD). The establishment of a translationally relevant animal model to assess these dysfunctions is essential to the development of effective therapeutic approaches. Our objective is to identify translationally relevant biomarkers linking age-related cognitive decline with the development of the Abeta plaques and Tau pathologies in the baboon model.

Methods: We choose two groups of animals, adult baboons with 10 to 16 years-old and aged group >19 years old based on our findings these nonhuman primates (NHP) develop significant cognitive decline and AD-like pathologies at the age of 19 years and older. To detect biomarkers predictive of age-dependent cognitive decline, we performed a longitudinal and cross-sectional analysis of mRNA and miRNA-Seq expression, cytokine/chemokine expression profiles and Abeta, pTau levels using the NHP Luminex assays. The NHPs were initially subjected to behavioral screen measuring the baseline motor and cognitive performances using the object retrieval task and CANTAB. The general activity and sleep patterns were monitored through an accelerometer device.

Results: The baboons exhibited age-dependent cognitive decline. The analyses of brain surrogate proteinopathy markers suggest an age-related prominent inflammatory component associated with increased levels of total and phosphorylated Tau, amyloid beta 1-40 (Ab40) and amyloid beta 1-42 (Ab42). The immunohistopathological analyses demonstrated a widespread microgliosis response throughout the brain and surrounding Tau and Ab pathologies.

Conclusions: Aged baboons may offer a relevant AD/ADRD model bridging preclinical success and translation to the clinic.
The pathological modulation of vesicular monoamine transporter-2 (VMAT2) and dopamine metabolism, in Parkinson's disease, by alpha synuclein.

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Aims: Alpha synuclein has been implicated as the faulty protein underlying the death of dopaminergic neurons in the substantia nigra (pars compacta), in the midbrain, of patients with Parkinson's disease, PD, leading to decrease dopamine release. Over the years, there have been inadequate information on how alpha synuclein is involved in the pathologies of the disease, hence, this review looked into one of the major pathological mechanisms involving the vesicles, involved, in the packaging and release of dopamine at the synapse, alongside the metabolism of dopamine in the cytosol of the dopaminergic neurons.

Methods: The biology of the physiological role of alpha synuclein, its conformational polymerization, leading to the toxic aggregation, and the implications of the protein in PD pathology was insightfully studied and reviewed. All these helped in understanding the specific pathways of the alpha synuclein involvement in PD pathogenesis.

Results: Aggregated forms of alpha synuclein is observed to initiate the rapid reuptake of dopamine at the synapse, leading to increase pool of the neurotransmitter in the cytosol. There is also the inhibition of the packaging and sequestration of the dopamine into the synaptic vesicles, due to the interaction of the polymerized alpha synuclein with the vesicular monoamine transporter-2. The cumulative result is the metabolism of dopamine to toxic metabolites, in the cytosol, leading to production of oxidative radicals, affecting mitochondrial and causing neuronal cell death.

Conclusions: The monomers of alpha synuclein is of physiological importance, however, the aggregates of the protein is implicated to alter the normal neural communication leading to Parkinson's disease.
Aims: Synucleinopathies, such as Parkinson's disease, dementia with Lewy bodies and multiple system atrophy, are disorders characterized by the aggregation of the α-synuclein protein. Unfortunately, no curative treatments currently exist for these diseases. Well-validated models are therefore essential to assess the efficacy of disease-modifying treatments. In this context, new models have been developed, based on the seeding property of aggregated proteins, to initiate the pathology in specific brain areas and reproduce the progression of the disease. Accordingly, we generated a disease-relevant model of α-synuclein aggregation by injecting protein seeds in vulnerable brain regions of knock-in mice expressing human α-synuclein (hSNCA mouse model).

Methods: α-synuclein pre-formed fibrils (PFF) derived from recombinant human protein were injected in the striatum of hSNCA or hSNCA A53T mice. Before injection, the structure and the seeding activity of these PFF were characterized and validated by biochemical and cellular assays. Different experimental conditions were investigated to induce α-synuclein pathology in the mouse brain and validated by histological and biochemical techniques.

Results: The injection of optimized PFF preparations induced a robust α-synuclein pathology, measured by pS129 positive staining, in different brain regions of the mice. We have also shown that the appearance of α-synuclein pathology is accelerated in the hSNCA A53T mice. Finally, the model was validated using reference compounds targeting α-synuclein such as antisense oligonucleotides (Cole et al. 2021).

Conclusions: We have developed an inducible and accelerated model of α-synuclein aggregation. This model will be useful for assessing the efficacy of new therapeutic approaches designed to slow the progression of synucleinopathies.
Aims: In Parkinson’s disease (PD), one hypothesis is that the formation and propagation of alpha-synuclein (alpha-syn) insoluble amyloid structures drives the disease process. Preventing protein aggregation or promoting protein clearance thus provide interesting opportunities for pharmacological intervention. We therefore aimed to establish an in vitro system for modelling these mechanisms to further the understanding of pathological processes occurring in neurodegenerative diseases.

Methods: Embryonic mouse cortical cultures in 384-well format were generated and endogenous alpha-syn aggregation was induced at 6 or 10 days in vitro using human alpha-syn pre-formed fibrils. Endogenous alpha-syn aggregation and cell health was then quantified using immunocytochemistry and automated high content imaging and analysis. Following the development of a robust protocol for induction and quantification of alpha-syn aggregation, the model was validated using commercially available small molecule modulators of alpha-syn aggregation, as well as using lentiviral shRNAs for knock-down of genes known for their involvement in PD.

Results: The established high-capacity in vitro assay is sensitive and has sufficient reproducibility to enable detection of both negative and positive modulators of alpha-syn aggregation in a single point screening format. While most of the commercially available small molecules tested were only weakly potent modulators of alpha-syn aggregation, the knock-down of the PD-relevant genes SNCA, LRRK2, GBA and TMEM175 resulted in the expected effect on alpha-syn aggregation.

Conclusions: The presented in vitro model can be used for high-capacity screening to identify small molecule modulators and genes affecting alpha-syn aggregation as well as for detailed target validation studies.
Aims: The lack of alpha-synuclein aggregation cell-based assays that would allow the screening of hundreds of thousands of molecules is a drawback to get new therapies against Parkinson’s disease. The formation of aggregates in neurons in response to apoptosis induced by staurosporine has been well established in the literature. The aim of this study was the development of a fluorescent cell-based assay to identify compounds able to inhibit or modulate the alpha-synuclein aggregation induced by staurosporine.  

Methods: The human SH-SY5Y cell line was used to generate a fluorescent alpha-synuclein stable cell line. In order to induce alpha-synuclein aggregation, cells were treated with 100 nM staurosporine 1 hour. Images were acquired and analyzed with a High-Content Bioimager after cell fixation. Immunofluorescence study was also performed with an alpha-synuclein antibody. The screening of 20 compounds was performed by pre-treating the cells with the test compounds overnight before staurosporine incubation. A post-treatment assay was also performed during 48 h after the incubation with staurosporine. Compounds were assayed at 3 different doses in triplicates.  

Results: The addition of staurosporine was able to increase the number of aggregates over 3-fold. The presence of alpha-synuclein in the aggregates was verified by Immunofluorescence.  

Conclusions: The fluorescent alpha-synuclein aggregation cellular model has been adapted to High Content Screening (HCS). This cellular model can be used in drug discovery to search for pathological protein aggregation inhibitors or modulators.
AGGREGATE-SPECIFIC ALPHA-SYNUCLEIN PLA REVEALS OLIGOMERIC PATHOLOGY IN SYNUCLEINOPATHY MODELS AND HUMAN BRAIN TISSUE FROM PARKINSON’S DISEASE AND MULTIPLE SYSTEM ATROPHY

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Aims: We aimed to develop an alpha-synuclein proximity ligation assay (PLA) with unprecedented specificity towards small, pathological alpha-synuclein aggregates, usable in model systems (including alpha-synuclein over-expression models) and in brain tissue from patients with synucleinopathies. Furthermore, we sought to design an automated method for the analysis of PLA-stained tissue sections using machine learning, thereby facilitating large-scale studies of PLA pathology.

Methods: To improve the specificity of the proximity ligation assay, we conjugated the conformation-specific anti-alpha-synuclein antibody MJFR-14-6-4-2 to proximity ligation probes (Duolink, Sigma). Staining procedures followed manufacturer's protocols, with modifications to antibody dilution and incubation times for various models/tissues. To facilitate automated quantification of PLA signal, chromogenic images were segmented into their components using Trainable Weka Segmentation (FIJI), and a macro set up to compute total, neuronal, and non-neuronal PLA signal. Moreover, each neuron was analyzed individually to define nuclear and cytoplasmic PLA signals.

Results: Signal in the MJF-PLA directly corresponds to the generation of early-stage aggregates, as inhibition of aggregation lowers the PLA signal. Alpha-synuclein over-expressing mice show increased pathology compared to non-transgenic littermates, and the assay also detects pathology initiated by preformed fibrils. The MJF-PLA detects novel pathology in synucleinopathies in brain regions previously considered unaffected, while it does not label the hallmark Lewy bodies.

Conclusions: The MJF-PLA is a useful method to study early-stage pathology in synucleinopathies and models thereof, especially combined with automated strategies for data analysis. With its preference for oligomeric pathology, the PLA might prove particularly relevant in efforts to limit disease progression.
Aims: Reliable and broad-range quantification of pS129-alpha-synuclein-positive inclusions of Lewy bodies (LB) and Lewy neurites (LNs) is a necessity in Parkinson’s disease research as these are present in both sporadic and familial forms of the disease in patients. The current methods of quantification for these aggregates include interobserver variation-prone stereology and automated image analysis methods, which are limited in their ability to reliably recognize the differences between LBs and LNs in the brain sections. In contrast to these methods, convolutional neural network (CNN) algorithms provide high performance in pattern recognition, making them an ideal platform for automated analysis of LBs and LNs.

Methods: Free-floating sections from mouse brain were immunostained with pS129-alpha-synuclein antibody and avidin-biotin-complex peroxidase technique. Sections were counterstained with hematoxylin to visualize cell nuclei. Digital whole-slide images (WSI) of brain sections were acquired with 3D HISTECH digital slide scanner at 0.22 μm/px resolution and uploaded to Aiforia Create® cloud platform for supervised CNN training.

Results: CNN algorithms were trained in Aiforia Create® platform to automatically detect intracellular LBs and LNs in WSIs of mouse brain sections and later validated against human counting to show the correlation and accuracy. The algorithm measured the length, width, area, and location for individual LNs, and total amount of LBs.

Conclusions: The combination of cloud-computing and CNN-based detection of pS129-alpha-synuclein immunoreactivity enables quantitative and reproducible high-throughput analysis of LNs and LBs in mouse brain. Provided the samples can be successfully stained with p129S and hematoxylin, algorithms enable quantifiable data of a variety of Lewy pathologies.
POSTERS

SWIMMING EXERCISE REDUCES NATIVE α-SYNUCLEIN PROTEIN SPECIES IN A TRANSGENIC C. ELEGANS MODEL OF PARKINSON’S DISEASE.

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Aims: Exercise has been historically recommended to prevent many disease conditions. Intense exercise in particular, has been shown to be beneficial for Parkinson’s disease (PD) — stopping and even reversing symptoms in some patients. Recent research in mammalian animal models of Parkinson’s have shown that exercise affects α-synuclein aggregate species, considered to be a hallmark of PD. However, the exact changes in native α-synuclein protein species after exercise and the downstream effects of exercise upon the health of the animals remains unclear. Here, we have aimed to understand the effect of exercise upon native human alpha-synuclein protein species in a transgenic C. elegans worm model of Parkinson’s disease.

Methods: We have utilized a swimming protocol for C. elegans worms and performed analysis of the effect of exercise upon native human α-synuclein protein species using Blue Native Page gels. Results were confirmed via confocal studies, and downstream effects of exercise and food restriction were observed via thrashing assays.

Results: Here we show that a period of swimming exercise (Ex) — 15-20 mins — dramatically reduces several native human α-synuclein protein species in the NL5901 C. elegans worm model of Parkinson’s. Exercise on Day 1 of adulthood was found to improve motor function measured by the thrashing rate of worms on Day 2 and Day 4 when compared to both control (untreated) and food restricted (FR) worms. Moreover, exercised worms show smaller α-synuclein::YFP puncta on average than food restricted worms.

Conclusions: Here we show that exercise reduces native human α-synuclein levels independent of food restriction in C. elegans.
A HUMAN IPSC DERIVED NEURONAL SCREENING ASSAY, BASED ON ALPHA-SYNUCLEIN SEEDING AND AGGREGATION, TO SCREEN FOR DRUGS FOR PARKINSON’S DISEASE

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Aims: Parkinson’s is a multi-systemic alpha-synucleinopathy, characterized by phosphorylation, misfolding, and abnormal accumulation of a-synuclein resulting in the death of the midbrains dopaminergic neurons. Although extensively studied, pathogenetic mechanisms are, so far, unknown. Human in vitro disease models based on dopaminergic neurons differentiated from iPSCs have demonstrated higher a-synuclein protein levels and increased phosphorylation of a-synuclein at serine residue 129 (pS129) compared to controls. Interestingly, seeding with recombinant a-synuclein fibrils have also been shown to lead to an increased pS129 phosphorylation with a subsequent aggregation of endogenous a-synuclein. Here we present a robust cell model for a-synuclein seeding with a resulting intracellular a-synuclein pS129 phosphorylation and aggregation in a screening suitable setup.

Methods: Gene-editing was employed to establish an iPSC line with doxycycline inducible alpha-synuclein expression. iPSC lines were differentiated to neurons using a modified version of a previously published neuronal differentiation protocol. Recombinant wildtype alpha-synuclein expressed in E. coli, was used to generate seeding fibrils. pS129-synuclein and pS129-pS129 aggregation alpha-synuclein FRET assays was used for analysis.

Results: First, a-synuclein aggregation was shown to increase with increasing concentration of doxycycline e.g. increasing alpha-synuclein expression, without measurable effect on cell survival. When exogenic fibrils are added to differentiated neurons overexpressing a-synuclein, we demonstrate an increased intracellular pS129 phosphorylation and seeding of intracellular a-synuclein aggregation using a-synuclein specific FRET-based assay.

Conclusions: Our model use doxycycline induced alpha-synuclein expression to generate a humanized seeding model. The model exhibits two fundamental characteristics for target validation in pharmacological research: a high reproducibility and a remarkable versatility for throughput studies.
DEVELOPING A CORRELATIVE CONFOCAL LIGHT AND ELECTRON MICROSCOPY PIPELINE FOR POST-MORTEM HUMAN BRAIN

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\textbf{Aims:} Correlative Light and Electron Microscopy (CLEM) is a powerful technique that combines light microscopy (LM) and EM to extract ultrastructural information from biological samples. Previous CLEM methods performed LM directly on ultrathin resin-embedded sections allowing easy correlation and unbiased identification of Parkinson’s pathology. However, in this pipeline, samples were randomly cut without prior knowledge of the spatial distribution of pathology within. Therefore, an unknown amount of pathology could be missed. To overcome this limitation, we developed a method to correlate confocal microscopy images in free-floating post-mortem human brain sections, with their ultrastructural details by EM after resin-embedding.

\textbf{Methods:} Fluorescent immunolabeling was performed on 40 microns-thick free-floating chemically fixed brain sections from Parkinson’s Disease patients and imaged using confocal laser scanning microscopy. The same sections were then processed for EM using en bloc staining and resin-embedding.

\textbf{Results:} We created computational fluorescent maps of alpha-Synuclein inclusions and their cellular context within free-floating sections. After EM processing the correlation of these maps in the resin-embedded samples was error-prone due to loss of visual markers and sample morphological changes. By utilizing programmable laser ablation, we achieved unprecedented cutting accuracy, minimizing correlation errors between the computational and physical ROIs. Sample damage from the laser was limited to a 10-30 microns region around the sample border, preserving the internal tissue ultrastructure.

\textbf{Conclusions:} With confocal microscopy we specifically mapped ROIs and using programmable laser ablation we improved the correlation reliability of the CLEM pipeline to efficiently target pathological lesions in post-mortem human brain.
A NEW CELLULAR MODEL TO STUDY A-SYNUCLEIN AGGREGATION, SEEDING AND PHOSPHORYLATION

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Aims: We aimed to develop a reliable cellular model to test and validate events of Lewy body (LBs) formation and maturation, to be used as a platform for screening candidate drugs for Parkinson’s disease (PD) and other synucleopathies. LB formation is a complex process that has recently been proposed as, rather than simply α-syn fibrillization, a major driver of PD pathogenesis. The growth and maturation of LB involves an intricate relationship between α-syn fibrillization, posttranslational modifications, and interaction with membranous organelles.

Methods: α-syn pre-formed fibrils (PFF) were produced from recombinant α-syn obtained from E.coli and aggregated according to established protocols. Structural features and seeding capacity were characterized by biophysics techniques. cell line was developed by INNOPROT, and puncta quantification was performed with CellProfiler.

Results: Using a transgenic cell line SH-SY5Y-α-syn-RFP that stably overexpresses α-syn-RFP, we quantified the number and size of α-syn-RFP aggregates after the addition of recombinant α-syn PFF. We demonstrate the presence of two population of α-syn-RFP aggregates. One of them that could be revealed with phospho-α-syn (S129) antibodies located close to plasma membrane, and a non-phosphorilated pool revealed positive for ThS staining, widely distributed throughout the cytoplasm.

Conclusions: The cellular model here presents advantages for the study of α-syn aggregation, seeding, and LD formation in cellula: i) a single antibody is needed to monitor two different events in the cells; ii) the seeding process can be followed in real time; iii) exogenous and endogenous α-syn can be discriminated.
POSTERS

INSIGHTS IN EARLY STAGE OF DIMERISATION OF ALPHA-SYNUCLEIN WILD-TYPE AND MUTANTS FROM MOLECULAR DYNAMICS SIMULATIONS

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Aims: In vitro studies showed that alpha-synuclein aggregates in cylindrical structures (fibrils), comprising hundred to thousands proteins, with polymorphic cross-beta-sheet conformations. The structures of the prefibrilar alpha-synuclein oligomeric species, formed at the early stage of aggregation, remain however poorly understood. Here, we aim to decipher the early stage of formation of alpha-synuclein dimers for wild-type (WT) and mutants (A30P, A53P, and E46K). Understanding the alpha-synuclein pre-fibrilar aggregation stage may inspire new therapeutic approaches.

Methods: We performed high-performance in silico molecular dynamics simulations for wild-type (WT) and mutants (A30P, A53P, and E46K) of alpha-synuclein with a coarse-grained model (United RESidue force field). All structures of alpha-synuclein were extracted from replica exchange molecular dynamics trajectories. Each trajectory was started with 2 fully-unfolded monomers separated by a distance of 25 Å.

Results: Alpha-synuclein dimers with fibril native contacts represent only a few per cents of all the conformations simulated, whereas the other structures are disordered. We identified two principal segments of the alpha-synuclein sequence with an higher propensity to aggregate in the early stage of dimerisation: residues 35-65 and residues 75-95. The transient alpha-helices (residues 53-65 and 73-82) of alpha-synuclein monomers are destabilized by A53T and E46K mutations which favors the formation of fibril native contacts whereas the alpha-helix 53-65 prevents the propagation of the native contacts along the sequence for the WT in the early stages of dimerisation.

Conclusions: Present results indicate segments of the amino-acid sequence of WT and mutants of alpha-synuclein relevant for dimerization which could be targeted by drugs.
Aims: Among the various conformations of alpha-synuclein, prefibrillar oligomeric species are proposed to be more toxic than fibrils. However, due to the heterogeneous and metastable nature, characterization of alpha-synuclein oligomeric species has been challenging. In this study, we characterized distinct off-pathway alpha-synuclein oligomers in vitro in the presence of dopamine (DA) and lipid peroxidation products 4-hydroxy-2-nonenal (HNE) and 4-oxo-2-nonenal (ONE).

Methods: We compared and characterized their structural, biophysical and functional properties by a combination of thioflavin-T (Th-T) fluorescence, electron microscopy, Circular Dichroism, Congo red binding assay, in vitro seeding assay, stability, toxicity in cells and their usability as calibrators in ELISA.

Results: We found that, though DA, HNE and ONE induces alpha-synuclein to form high molecular weight oligomeric species, the ONE oligomers were more stable towards treatment with SDS, urea, and temperature. The secondary structure analysis using circular dichroism, Th-T and Congo red binding assay revealed that only HNE and ONE oligomers contain beta-sheet content. In in vitro seeding assay, both DA and ONE oligomers significantly accelerated the aggregation of alpha-synuclein monomers after 48 h of incubation, whereas the HNE-oligomers had an effect only at 72 h. Furthermore, all oligomeric preparations were found to seed the aggregation of alpha-synuclein monomers and increase the cytotoxicity when added to SH-SY5Y cells. Both HNE and ONE alpha-synuclein oligomers can be used as a calibrator in a sandwich-based ELISA.

Conclusions: Despite formation of oligomers in an off-pathway assembly, the oligomeric preparations were found to be structurally distinct with various degree of stability and toxicity.
Aims: α-synuclein is involved in synaptic vesicle processing. Abnormal aggregates of α-synuclein form Lewy Bodies in neurons, a hallmark feature of neurodegenerative synucleinopathies such as Parkinson’s Disease and Lewy Body Dementia, and glial cytoplasmic inclusions seen in Multiple System Atrophy. Missense mutations in the gene encoding α-synuclein cause rare familial autosomal dominant forms of synucleinopathies. Like Prion Protein and tau, a growing body of literature has shown aggregates of α-synuclein form unique “strain” structures that replicate in living systems. The diversity of α-synuclein strains may propagate pathology and could account for the diversity of associated clinical and neuropathological syndromes. However, it is not understood which regions of α-synuclein drive aggregation.

Methods: We performed a saturation mutagenesis screen in a mammalian reporter cell line to reveal key regions of α-synuclein required for aggregation and fibril formation. To understand what specific interactions are responsible for these processes, we employed cross-linking coupled with mass spectrometry on α-synuclein mutants.

Results: Saturation mutagenesis revealed four domains in α-synuclein that are required for fibril formation. α-synuclein truncations increased its seeding capacity in biosensor cells, suggesting the presence of regulatory elements in the protein’s termini which may control the aggregation process. Furthermore, cross-linking coupled with mass spectrometry let us identify hotspots of electrostatic interactions that transiently stabilize interactions with regulatory elements.

Conclusions: α-synuclein contains four domains required for aggregation. However, that process might be limited by the regulatory elements localized in the termini. We propose a model in which the N-terminus of α-synuclein regulates the fibrillization propensity of its aggregation-prone core.
CEREBRAL ISCHEMIA INDUCES PARKINSON'S DISEASE-LIKE SYMPTOMS AND PATHOLOGY IN MICE

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Aims: The etiology of Parkinson's disease (PD) is not well understood and is associated with genetic predisposition and advancing age. Epidemiological findings suggest that patients with cerebral ischemia have a higher risk of developing PD, but these findings lack mechanistic evidence. We investigated the effects of cerebral ischemia on pathogenesis in hemizygous TgM83⁺⁻ mice that express human α-synuclein with the A53T mutation without developing neurologic disease or neuropathology for more than 600 days.

Methods: We induced a transient focal ischemia by middle cerebral artery occlusion in 8-week-old TgM83⁺⁻ mice and observed their behavior and health status up to one year later. Groups of mice were killed at different time points after surgery for pathological analysis of their brains.

Results: Motor deficits first appeared 6 months after focal ischemia and worsened until 12 months afterward. We observed ischemia-related neuronal loss in the infarct region and astrogliosis and microgliosis, indicative of an inflammatory response, which was most pronounced at 14 days after surgery. Infarct volume and inflammation decreased steadily in size and severity until 6 months after surgery, after which neuronal loss and inflammation began to increase again. Surprisingly, these changes were accompanied by continuous aggregation of α-synuclein and late loss of dopaminergic neurons in the substantia nigra, which we observed 12 months after surgery. Control animals that underwent sham surgery without middle cerebral artery occlusion showed no signs of neuropathology or disease.

Conclusions: Our results establish a mechanistic link between ischemic stroke and PD and provide an animal model to study possible interventions.
Aims: Single-nucleotide polymorphisms (SNP) in the leucine-rich repeat kinase 2 (LRRK2) locus are consistently associated with risk of developing Parkinson’s disease (PD). We recently found that a PD-associated risk SNP (rs76904798) in the LRRK2 locus is also associated with cerebrospinal fluid levels of granulins (PGRN), glycoprotein nmb (GPNMB), Ectonucleoside triphosphate diphosphohydrolase-1 (ENTPD1) also known as CD39 and Cathepsin B (CTSB). Yet, functional validation in cellular models is lacking.

Methods: Human monocytic cell line (U937) was differentiated (dU937) into macrophages to test the effect of LRRK2 on CTSB, CD39, GPNMB, PGRN and alpha-synuclein (aSyn). LRRK2 kinase activity and expression level were modulated using pharmacological inhibitors and lentivirus-mediated overexpression. Protein levels were assessed using western blotting and ELISA. dU937 cells were treated with human aSyn pre-formed fibrils (haSyn PFFs).

Results: Overexpression of LRRK2 increased the phosphorylation of LRRK2-Ser935 and Rab10-T73 and intracellular levels of lysosomal proteins CTSB and Saposin D without affecting LAMP1 or M6PR. LRRK2 also reduced extracellular CD39, PGRN and GPNMB levels. Furthermore, LRRK2 overexpression reduced the levels of intracellular endogenous aSyn dimers. HaSyn PFF treatment induced a time-dependent reduction of CTSB and GPNMB levels, increased PGRN levels and altered intracellular aSyn processing pattern. Also, LRRK2 kinase inhibition decreased intracellular CTSB levels. LRRK2 interacts directly with PGRN in cells and in human brain.

Conclusions: Our data supports a pleotropic role of the LRRK2 locus as a genetic modifier of PD-associated proteins. Our biochemical data show that LRRK2 modify lysosome-associated proteins and regulate aSyn proteostasis.
LRRK2 PROTECTS IMMUNE CELLS AGAINST ERASTIN-INDUCED FERROPTOSIS

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Aims: Ferroptosis is an iron-dependent regulated cell death pathway that is characterized by excessive lipid peroxidation, and is implicated in neurodegenerative diseases including Parkinson's disease (PD). Mutations and increased activity of leucine rich-repeats kinase 2 (LRRK2) are linked to both familial and idiopathic PD. LRRK2 is highly expressed in immune cells including macrophages. However, the function of LRRK2 in the immune cells is still elusive. In this study, we aim to investigate the effect of LRRK2 and its kinase function on ferroptosis in immune cells.

Methods: Murine macrophages (LRRK2 parental RAW 264.7 (WT) cells and LRRK2 KO RAW 264.7 (KO) cells) were treated with erastin (ferroptosis inducer) with/without the LRRK2 kinase inhibitor (MLi2). Changes in cell metabolic activity, morphology and viability were determined by MTT, xCELLigence (real-time cell impedance system) and flow cytometry with Annexin/Pi, respectively. Lipid peroxidation was determined via flow cytometric measurements using BODIPY® 581/591 C11 sensor.

Results: LRRK2 WT cells are more resistant to erastin induced ferroptosis compared to the KO cells; indicated by higher metabolic activity and cell viability. In addition, lipid peroxidation is significantly elevated in the LRRK2 KO cells compared to the WT cells. Pretreatment of the WT cells with LRRK2 kinase inhibitor increases the cell sensitivity to erastin depicted by lower cell metabolic activity and higher lipid peroxidation compared to the WT cells.

Conclusions: These results indicate a protective effect of LRRK2 against erastin-induced ferroptosis in macrophages and point towards the importance of the kinase function of LRRK2 in this protective mechanism.
Aims: Clinical and pathological overlap between PD and FTD has become apparent in recent years. An emerging body of data demonstrates the convergence of PD- and FTD-associated genes on the lysosome. LRRK2, GRN and GBA, have all been implicated in lysosomal function. How LRRK2-/-GRN-/-GBA-associated phenotypes intersect and converge at the lysosome in myeloid cells is still not understood. Here we aimed to characterise lysosomal function and GBA activity in peritoneal macrophages (pMacs) of Grn-/- mice and G2019S or R1441C Lrrk2 KI mice.

Methods: pMacs from GRN-/- mice, G2019S or R1441C Lrrk2 KI mice and B6 controls were plated and stimulated, ex-vivo, with 100U IFN-γ or 100ng/mL LPS +/- 100nM MLii2 for 18h prior to flow cytometry

Results: Small pMacs from GRN-/- mice displayed increased GBA index relative to B6 controls. Large pMacs from these mice, however, displayed decreased GBA index. LRRK2 kinase inhibition successfully rescued these phenotypes. This was accompanied by a decrease in antigen presentation in pMacs from GRN-/- mice. Conversely, large pMacs from young G2019S- and R1441C Lrrk2 exhibited increased antigen presentation relative to B6, whilst pMacs from aged R1441C Lrrk2 KI animals displayed decreased antigen presentation, lysosomal function and GBA index.

Conclusions: This data indicates an interaction between GBA, GRN and LRRK2 at the lysosome in myeloid cells of models of FTD and PD. If and how alterations in lysosomal function subsequently leads to immune dysregulation in these models is yet to be determined. This is of high importance in order understand how lysosomes may be targeted for immune-therapies.
OXIDATION OF CYS106 OF DJ-1 DEFINES ITS PROTECTING FUNCTIONAL ROLE IN GLYOXAL HOMEOSTASIS AND NEURODEGENERATION

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Aims: The oxidation of Cys106 of DJ-1 has been implicated in defining the functional role of DJ-1 in protecting against neurodegeneration. DJ-1 is a glyoxalase and have been associated with alleviating toxicity due to glyoxal stress. In neurodegenerative diseases glyoxal homeostasis is thought to be impaired. Our objectives are to elucidate the impact of Cys106 oxidation on the enzymatic functions of DJ-1 in vitro and cell models of glyoxal stress to understand the mechanism by which DJ-1 can be involved in glyoxal detoxification.

Methods: Biochemical, biophysical, proteomics and cell biology techniques were applied. To explain the observed differences in enzymatic activities between redox states of Cys106 of DJ-1 from a structural point of view, we solved their crystal structure using similar crystallization conditions.

Results: Significant role of DJ-1 in glyoxal homeostasis was confirmed in DJ-1 KO and overexpressed cell lines. The oxidation of the catalytic Cys106 of DJ-1 abolishes its enzymatic activities in vitro and cell models. Native DJ-1 alleviates GO/MGO cell toxicity via its glyoxalase activity, while DJ-1 with oxidized Cys106 does not. Oxidation of Cys106 caused significant enough conformational perturbations to alter the active site of DJ-1 resulting in a change in the reactivity, redox and acidic character of the Cys106 side chain.

Conclusions: DJ-1 can protect from glyoxal caused toxicity in disease. While the oxidation of Cys106 of DJ-1 leads to the loss of its enzymatic activity, we postulate that it also results in a gain of new protective functions against disease driving toxic processes such as high oxidative stress and protein aggregation.
Aims: Cys106 of DJ-1 protein is a critical residue responsible for controlling a variety of its functions due to its sensitivity to oxidative stress. The total concentration and/or the ratio of DJ-1 redox isoforms (C106-SO₂H/-SO₃H) may be an indicator of disease onset and progression. Therefore, our aim is to develop novel antibodies and ultimately ELISAs that enable the quantification of native and oxidized states of Cys106 of DJ-1.

Methods: BioRad's synthetic monoclonal Human Combinatorial Antibody Library (HuCal PLATINUM) based phage display approach was applied in combination with guided selection strategies using recombinant DJ-1 protein and DJ-1 derived peptide-conjugate antigens, in which competition with similar antigen could drive specificity. DJ-1 KO and WT SH-SY5Y neuroblastic cell lysates were utilized during ELISA validation experiments.

Results: The HuCal screen identified 25 antigen binding fragments (Fabs) with varying selectivity and affinity towards the redox isoforms of Cys106 of DJ-1 that were further characterized in multiple ELISA formats. Rational pairing of Fabs lead to the development of sandwich ELISAs specific for Cys106 isoforms of DJ-1 level from biofluids.

Conclusions: We developed novel ELISAs that may be used for the quantification of the individual redox states of Cys106 of DJ-1 from cell lysates and biofluids, which enables the assessment of their levels as
a potential biomarker for Parkinson's disease and related neurodegenerative diseases.
IMPACT OF DJ-1 MODIFICATION BY OXIDIZED DOPAMINES ON ITS AND STRUCTURAL STABILITY, ENZYMATIC AND CHAPERONE FUNCTIONS

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Aims: Oxidized dopamines (DAs) play an important role in the neurodegenerative processes of Parkinson's disease (PD). Our goal was to investigate the complex reactions between DA/oxidized DA species and DJ-1 and elucidate their effects on its enzymatic activity, structure, stability, chaperon activity and the oxidation state of Cys106, a critical catalytic residue.

Methods: Oxidized DAs were prepared in situ from DA and 6OHDA by using tyrosinase, while hydrogen peroxide was quenched with catalase. ROS level was determined with FOX I assay. Intact protein mass spectrometry, trypsin-based proteomics were applied to characterize the covalent modifications on DJ-1 triggered by oxidized DAs. The kinetics of the inhibition of DJ-1 enzymatic function was monitored using the esterase and glyoxalase activity assays. The impact of oxidized DA on the stability and structure of DJ-1 was assessed using CD, DSF and ThioT aggregation assays.

Results: Oxidized DAs can react with both the Cys53 and Cys106 residues of DJ-1 resulting in a mixture of various dopamine quinone modified protein species. Modification at Cys106 of DJ-1 results in a loss of its enzymatic functions and increases the propensity of DJ-1 to aggregate. Hydrogen peroxide generated during autooxidation of DAs competes for Cys106 to form Cys106-SO₂H DJ-1.

Conclusions: Oxidized DAs can modify the structure and activity of DJ-1 resulting in the loss of its enzymatic activities, destabilization of its homodimer structure and triggering ThioT positive aggregate formation. This may be a prevalent effect in dopaminergic neurons in PD that contributes to the onset and
progression of the disease.
IN-DEPTH MOLECULAR PROFILING OF PARKINSON'S DISEASE USING ADVANCED MIDBRAIN ORGANOID MODELS

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Aims: Novel models for the use in Parkinson’s disease (PD) drug development are needed and midbrain organoids may fulfil these needs. Patient derived midbrain organoids are closer to real life patients, fully human, can be cultured for extensive periods and offer significant advantages over 2D culture models.

Methods: Combining midbrain organoid models with in-depth molecular profiling on protein, phosphorylation and ubiquitination level, molecular mechanisms for PD and new potential drug targets can efficiently be identified limiting the use of animal models while being closer to the patient situation. Antibody Microarrays for immuno-based protein and post-translational modification analysis developed by Sciomics cover key pathways and secreted proteins. With minimal sample consumption in supernatant or cells a spectrum of more than 1,300 proteins as well as the respective phosphorylation and ubiquitination is detected covering signalling molecules, transcription factors, markers for apoptosis, oxidative stress, cell surface markers and cytokines.

Results: In the presented study PD midbrain organoids and culture supernatants from midbrain organoids were analysed on protein, phosphorylation and ubiquitination level. The results demonstrate the feasibility of such combined analysis and have yielded insights into the PD biology. Important proteins such as GDF15 and DKK3 were identified in the supernatants as well as matching phosphorylation and ubiquitination levels of TBB3 in both midbrain organoids and supernatant.

Conclusions: These results show the great potential of advanced models combined with highly multiplexed protein and post-translational modification analysis to generate knowledge as well as to speed research in PD as well as other neurodegenerative diseases.
MULTI-OMICS CHARACTERISATION OF PARKINSON’S DISEASE DEVELOPMENT IN IPSCS DOPAMINERGIC DIFFERENTIATION

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Aims: Parkinson’s disease (PD) is a neurodegenerative disease that predominantly affects dopaminergic (DA) neurons, which are progressively lost in the midbrain substantia nigra. No cure for PD has been found so far, as the mechanism of onset and progression of this disease remain elusive. Most of PD cases are thought to be idiopathic, while only 10% of patients display genetic mutations. Although these mutations have been strongly associated with mitochondrial activity, a comprehensive understanding of the underlying mechanisms is still lacking.

Methods: In this project, we address this gap by investigating the effect of PD-related mutations, with a focus on the ones affecting PINK1 gene, in patient and control subjects-derived induced Pluripotent Stem Cells (iPSCs) during their differentiation into DA neurons. First, we established an optimised protocol for iPSCs differentiation to generate high-quality DA neurons. We then monitored the differentiation dynamics by single-cell RNA-sequencing at different time points to identify mutation-induced developmental impairments. Proteomics and metabolomics assays have also been performed in order to have a more meaningful perspective of the molecular dynamics of PD.

Results: This multi-omics analysis allowed us to highlight mechanisms of neuronal development and identified a subset of genes potentially driving neurodegeneration. Since all these genes converge on mitochondrial activity and identify a core network of mitochondrial dysfunction in PD, we could formulate the hypothesis that PINK1 mutations might also severely affect other PD-related genes.

Conclusions: The integration of these findings could pave the way for a more exhaustive perspective on PD and may aid the development of personalized therapies.
Aims: A feature of Parkinson’s disease (PD) pathology is the frequent accumulation of iron correlating with motor symptom severity, but the mechanisms that lead to iron dyshomeostasis in PD remain poorly understood. Recently, we demonstrated dysregulation of transferrin trafficking in cellular models of LRRK2 expression and iron accumulation in the striatum of LRRK2 knockin mice under pro-inflammatory conditions (Mamais et al, 2021). While these data support a role of LRRK2 in cellular iron, the influence of cell autonomous LRRK2 mutation in neuronal iron homeostasis remains unexplored. Our objective is to interrogate iron homeostasis in human neurons and parental iPSCs from G2019S LRRK2 PD, R1441C/G LRRK2 PD and healthy controls.

Methods: Iron homeostasis was assessed in human neurons and iPSCs from controls, G2019S LRRK2 and R1441C/G LRRK2 PD patients (NINDS, PPMI). iPSCs were differentiated into cortical neurons by NGN2 expression and analyzed 21 DIV. Iron content was assayed by high-content imaging using selective Fe²⁺ imaging probes, and levels of iron-related factors were assessed by immunoblotting.

Results: Our data showed a significant increase in intracellular iron in human neurons and as well as parental iPSCs from G2019S LRRK2 PD and R1441C/G LRRK2 PD patients compared to WT controls. Furthermore, a concomitant upregulation of ferritin and DMT1 was observed, highlighting alterations in cellular iron storage and transport with LRRK2 mutations.

Conclusions: Our data highlight impairment of iron homeostasis driven by LRRK2 mutations in physiological and disease-relevant model systems. The role of LRRK2-driven endolysosomal alterations as well as Rab GTPase signaling in these phenotypes is currently being explored.
**LRP10 CELL-TO-CELL TRANSMISSION AS A POSSIBLE PATHOGENIC MECHANISM IN PARKINSON’S DISEASE AND DEMENTIA WITH LEWY BODIES**

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**Aims:** Pathogenic variants in LRP10 have been identified in patients with Parkinson’s disease (PD) and dementia with Lewy bodies (DLB). How variants affect LRP10 protein function remains unknown. Interestingly, LRP10 expression in healthy brains is almost exclusively limited to non-neuronal cells, e.g. astrocytes and neurovasculature. However, in PD and DLB patients LRP10 localizes to Lewy bodies in neurons. In this work, we aim to study cell-to-cell transmission of LRP10 via extracellular vesicles (EVs) and the role of LRP10 in regulating EVs secretion.

**Methods:** Western blotting, immunocytochemistry, transmission electron microscopy and EVQuant were used in isolated EVs from media and lysates from HEK-293T cells, fibroblasts and iPSC-derived astrocytes from c.1424+5G>A LRP10 variant-carrying patients and healthy individuals.

**Results:** Wild-type LRP10 is exclusively secreted via EVs in HEK-293T cells, control fibroblasts and control iPSC-derived astrocytes. In contrast, patient-derived astrocytes carrying the c.1424+5G>A LRP10 variant were found to secrete an aberrant high-molecular weight species of LRP10 in EV-free fractions. Strikingly, overexpression of the c.1424+5G>A LRP10 variant lead to the abnormal secretion of LRP10 products of different sizes in the EV-free compartment. Finally, wild-type LRP10 overexpression was found to induce changes in EVs levels and composition.

**Conclusions:** In this study, we show that wild-type LRP10 is secreted via EVs, and patient-derived LRP10 forms are secreted aberrantly in the EV-free fraction. This work could potentially explain the non-cell autonomous origin of LRP10 in Lewy bodies, which could be an important disease mechanism in PD and DLB.
INTRAGASTRIC ADMINISTRATION OF LOW DOSE ROTENONE POST COLITIS ACCELERATE BRAIN NEUROPATHOLOGY AND MOTOR IMPAIRMENT IN PARKINSON’S DISEASE

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Aims: The contribution of gastrointestinal (GI) inflammation and local exposure of neurotoxin in the gut, offers the most in-depth explanation of Parkinson’s disease (PD) etiopathogenesis through abnormal accumulation and spreading of alpha-synuclein (αSyn) aggregates from the gut to the brain. This study was therefore designed to assess the exacerbation of proinflammatory intestinal milieu in a progressive mouse model of PD.

Methods: To induce chronic colitis, 10 months old C57BL/6 mice were treated with 1% Dextran Sodium Sulphate (DSS). After colitis-induction, animals received a low dose of intragastric rotenone (which was undetectable in systemic blood or brain) for the next 8 weeks, followed by testing for Parkinsonian behavior and GI phenotypes of inflammation. At the end of the 8th week, the intestine, brain stem, and midbrain tissue were isolated and analyzed for the presence of misfolded αSyn, inflammatory markers, and dopaminergic neuronal loss in substantia nigra, enteric neurons, and dorsal motor nucleus of the vagus (DMV).

Results: We found that intragastric administration of rotenone after colitis significantly decreased colon length as well as increased expression of inflammatory markers (CCl2, TNF-α, IL-1β, IL-6) in the colon and striatum. Gut inflammation in a mouse model of PD also disrupted GI architecture and caused loss of tight junction proteins (ZO-1, claudin1, occludin) in the colon compared to control mice. Gut inflammation accelerated the onset of motor dysfunction and significantly increased the expression of GFAP, phosphorylated αSyn in the cortex, and striatum.

Conclusions: Our findings suggest a critical role of intestinal inflammation in the initiation and progression of PD.
ACCUMULATION OF ALPHA SYNUCLEIN IN THE MOUSE RETINA AFTER MOUSE ALPHA SYNUCLEIN PRE-FORMED FIBRILS INTRAVITREAL INJECTION

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Aims: So far, the mechanisms underlying aggregation and spreading of alpha synuclein in the retina have not been addressed in detail. On this work, we studied the accumulation of α-synuclein in the retina and the brain of wild type mouse after the intravitreal injection of mouse α-synuclein (mPFF).

Methods: To characterize the aggregation process in the retina and later propagation, we injected intravitreally 5 ug of mouse pre-formed fibril (mPFF) in wild type mice and examined the expression of alpha synuclein in retinal lysates one and two-months after injection. In addition, we performed histological analysis of the retina and brain areas connected to the visual pathway to evaluate inflammatory and glial response, pathogenic alpha synuclein localization and cell loss. Cytokine expression was measured using RT-PCR.

Results: We observed that mPFF injection into the vitreous body led to aggregation of α-synuclein in the retina causing glial activation, inflammation and loss of tyrosine hydroxylase activity after two months without affecting retinal ganglion cells. In addition, we observed the presence of truncated alpha synuclein. Although we found synuclein aggregates in optic nerve lysates, we did not find phosphorylated synuclein in optic related brain areas two-months after injection.

Conclusions: Similar as brain injection models, mPFF leads to accumulation of alpha synuclein in the retina and optic nerve, leading to activation of glial cells. However, no retina to brain propagation or brain inflammatory response was seen two-months after injection.
Aims: Alpha-synuclein (αSyn) is the pathological hallmark of the synucleinopathies. αSyn aggregates are localized in specific brain regions and may originate in the gastrointestinal tract and spreading to the brain. Accumulating evidence suggests that αSyn follows the same mechanism of seeding, self-propagation and cell-to-cell spreading of prions. Here, we want to assess if differences in structural and phenotypic features of αSyn aggregates are the cause of the heterogeneous nature of the synucleinopathies or different αSyn strains can be present in the same patient in different regions.

Methods: We compare the amplified αSyn aggregates derived from the isolation of brain regions (cerebellum, frontal cortex) and transverse colon of the same patients using a combination of different methods: (i) Real-time quaking-induced conversion (RT-QuIC) assay. (ii) Proteinase K (PK) enzyme digestion of amplified αSyn aggregates enzyme, followed by Western blotting (WB) and mass-spectrometry (MS). (iii) Cryogenic electron microscopy. (iv) Seeding of neuroblastoma cell line.

Results: We optimized RT-QuIC assay, with human samples as seeds. We amplified brain and transverse colon αSyn sarkosyl-insoluble aggregates from DLB, PD and MSA patients to generate larger, highly pure batches of human αSyn aggregates. After PK digestion on amplified material, differences in the WB bands pattern between cerebellum and frontal cortex of MSA patients were observed. We are now processing the other samples in WB, MS and Cryo-EM.

Conclusions: Structurally and biochemically describe different αSyn strains present in the frontal cortex, cerebellum and colon of a single patient and/or between patients with different synucleinopathies.
Aims: The overlap of clinical, neuropathological, and genetic features between lysosomal storage diseases (LSD) and neurodegenerative diseases has become more evident through diverse studies. Especially, the genetic association between LSD and neurodegenerative diseases such as synucleinopathy and tauopathy is focused in this study under the hypothesis that hypofunction of LSD gene mediates the propagation and accumulation of pathological proteins such as α-synuclein and tau, further influencing the pathogenesis and progression of neurodegenerative diseases.

Methods: RNA interference (RNAi) screening was performed by feeding the plasmids against LSD genes to Caenorhabditis elegans (C.elegans) bimolecular fluorescence complementation (BiFC) models of the transmission of α-synuclein or tau.

Results: The RNAi screening identified the common or disease-specific candidate genes, which have significant changes in the transmission of pathological proteins.

Conclusions: In accordance with the candidate genes, we expect to validate in knock-out cell models, and to further discover the pathological mechanism.
POSTERS

IN VITRO MODELLING OF PROGRESSIVE ALPHA-SYNUCLEIN AGGREGATION IN PRIMARY CORTICAL CULTURES USING A HIGH-CAPACITY MICROFLUIDIC CO-CULTURE PLATFORM

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Aims: Misfolded and aggregated alpha-synuclein (alpha-syn) within neurons of the brain are hallmarks in Parkinson’s disease. Several studies indicate that misfolded alpha-syn is released by neurons and taken up by recipient neurons where it acts as seeds to cause misfolding and aggregation of endogenous alpha-syn. The aim was to establish a cellular system for neuron-to-neuron spreading of alpha-syn pathology to further enable the study of the cellular processes involved.

Methods: A high-capacity microfluidic co-culture platform, immunocytochemistry and high-content imaging were used to assess aggregation of endogenous alpha-syn in transgenic mouse cortical neurons, overexpressing human alpha-syn. In detail, embryonic transgenic F28 mice cortical neurons were plated allowing axons to grow into a cell-free interconnecting well. At 6 DIV, recombinant fibrillated human alpha-syn seeds (PFFs) were applied to the cell-free axonal side. Cultures were maintained for up to 34 DIV before assessment of neuron-to-neuron spreading of alpha-syn.

Results: A subpopulation of neurons projected into the interconnected well and was able to take up the applied PFFs that subsequently induced endogenous alpha-syn aggregation. The aggregates appeared as perinuclear Lewy-body like accumulations and axonal speckles. The amount of neurons developing alpha-syn pathology was high in the cells whose axons was able to directly take up the applied PFFs. We however also provide evidence that cells not directly exposed to the PFFs developed alpha-syn pathology, indicating cell-to-cell spreading of alpha-syn aggregation in this model.

Conclusions: By using our microfluidic co-culture platform, we have established a model useful for studying the cellular processes important for cell-to-cell spreading of alpha-syn aggregation.
POSTERS

TIMECOURSE ASSESMENT OF PATHOLOGY FOLLOWING BILATERAL INOCULATION OF ALPHA-SYNUCLEIN PREFORMED FIBRILS IN C57BL/6 MICE

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Aims: We sought to improve on previously published Parkinson's disease models based on alpha-synuclein, by studying the pathology that develops over time post-inoculation uni- or bilaterally of in vitro generated mouse alpha-synuclein preformed fibrils into wild type mice.

Methods: C57BL/6J mice were inoculated with PFF either uni- or bilaterally following striatal stereotaxic cannulation. Behavior and immunohistochemistry were assessed 30, 60, 90 and 180 days post-inoculation (DPI).

Results: No significant motor deficits were observed following uni- or bilateral PFF striatal inoculation. PFF-injected mice displayed strong Lewy-body-like pathology with hyperphosphorylated alpha-synuclein aggregates spreading from the striatum to the substantia nigra (SN), amygdala and layer IV of the neocortex. Stronger pathology was seen in striatum of bilaterally inoculated mice at 30DPI and dropping by 50% at 60DPI onwards. In the SN, the drop occurred at later DPI reflecting loss of neurons in this region. In contrast, multimeric alpha-synuclein increased from 30 to 90DPI while not altered in the unilateral model 90DPI. Formed pathological aggregates seeding potency was confirmed ex vivo in primary neurons, being more prominent for the 30DPI group. Substantial neuronal loss observed in the SN of bilaterally injected mice at 60DPI, 30 days earlier than the unilateral model and in the striatum between 90 and 180DPI.

Conclusions: Inoculating PFF bilaterally into the striatum of wild type mice led to a substantial enhancement of pathology that was apparent as a much as 30 DPI with no impact in motor behavior readouts. Further investigations are required to characterize this model for testing disease-modifying therapies for Parkinson’s disease.
EVALUATE THE EFFECTS OF CMA MALFUNCTION INDUCED BY AAV-MEDIATED LAMP2A DOWNREGULATION IN DIFFERENT EXTRA-NIGRAL RAT BRAIN REGIONS, SUCH AS THE HIPPOCAMPUS

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Aims: Chaperone-mediated autophagy (CMA) is one of the major pathways for α-synuclein degradation. We have previously reported that CMA impairment results in the accumulation of aberrant α-synuclein within dopaminergic neurons of the substantia nigra (SN) and a dying-back axonopathy. This robust neurodegenerative phenotype may suggest a selective vulnerability of this region compared to others, less affected in Parkinson’s disease (PD). This study aims to investigate the effects of CMA malfunction in different PD-relevant extra-nigral brain regions, such as the hippocampus.

Methods: GFP-tagged adeno-associated viruses expressing shRNAs targeting the rate-limiting step of the CMA pathway, the transmembrane receptor LAMP2A or a scrambled control sequence, were unilaterally stereotaxically injected into the CA1 region of female rat hippocampus. At 2 months post-injection, we assessed the levels of α-synuclein and ubiquitin, as well as, the neuronal viability of transduced hippocampal neurons.

Results: CMA downregulation led to α-synuclein accumulation within cell bodies, accompanied by increased levels of perinuclear ubiquitin-positive puncta within transduced hippocampal neurons. In contrast to the robust neurodegeneration observed upon LAMP2A downregulation within the nigral dopaminergic neurons, no evident neuronal loss could be observed within the transduced hippocampal neurons.

Conclusions: Our data insinuate a relative resilience of the hippocampal neurons to CMA malfunction as compared to the dying-back axonopathy observed in the nigral neurons, at least 2 months post-injection. If this holds true it could, at least in part, explain the preferential affection of the SN in PD, and further validate the importance of a proper CMA function for the dopaminergic system integrity.
POLO LIKE KINASE 2 IS A TARGET OF PROTEASOMAL PROTEOLYSIS RESPONSIBLE FOR A-SYN PHOSPHORYLATION AND ITS REGULATION

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Aims: Parkinson’s disease (PD) is characterized by the accumulation of α-synuclein (αS) aggregates and pS129αS in the pathogenesis of α-synucleinopathy.
Methods: αS overexpressing transgenic mice.
Results: Here, we show that polo like kinase 2 (PLK2), a major kinase for generating pS129αS is a short-lived protein that is constitutively targeted by proteasomal proteolysis. Study reveals that PLK2 half-life is about 1h, mirrored by pS129αS levels in M17 cells. A proteasome inhibition by MG-132 or PS341 potentiates PLK2 level and increases pS129αS levels. Paradoxically, increase in PLK2 and pS129αS levels are accompanied by reduced total αS level, presumably by autophagic degradation. Proteasome inhibition on pS129αS and αS levels are blocked by a PLK2 inhibitor, Bl-2536. A genetic study of PLK2 shows induction of pS129-αS from overexpression of PLK2, and conversely SiRNA of PLK2 resulted in significant reduction of pS129-αS, indicating that S129 in αS is a specific target of phosphorylation by PLK2. In vivo, we hypothesize that pS129αS accumulates because of increase in PLK2 due to proteasomal dysfunction. Consistent with our hypothesis, αS mouse model (TgA53T) of α-synucleinopathy show that PLK2 level increases in neuron containing pathological aggregated form of pS129αS in symptomatic end-stage animal. Accumulation of PLK2 in TgA53T model is coincident with signs of proteasomal deficit as indicated by accumulation of degron-GFP in TgA53T neurons.
Conclusions: Collectively, our study support the view that induction of PLK2 is a compensatory response by neurons to degrade αS in presence of proteasomal dysfunction. However, this response is futile in α-synucleinopathy because of concurrent deficits in autophagy and lysosomes.
Aims: Mutations in GBA are the greatest numerical risk factor for Parkinson disease (PD), however, the pathological mechanisms underpinning this association are not understood. A lysosomal network, comprising progranulin (GRN), prosaposin (PSAP), and cathepsins B and D (CTSB/D), has been implicated in GBA-associated PD (GBA-PD). We aim to define this lysosomal network in both wild-type and PD cell models.

Methods: Using a novel protocol, we generated midbrain dopaminergic (mDA) neurons from healthy and SNCA mutant (p.A53T, p.G51D, and triplication) human iPSC lines. We measured the gene and protein expression of the lysosomal network at each developmental stage. In SHSY5Y cells, we used siRNA technology to genetically knockdown (KD) each network component to determine any interdependency of expression, as well as evaluate any effect on β-glucocerebrosidase (GCase) activity. We are currently generating iPSC-derived mDA neurons from GBA-PD (p.N370S) patients (N=3) to measure the activity and expression of the network components and compare these to unrelated controls and an isogenic control line.

Results: Compared to controls, the mDA neurons from the SNCA p.G51D line expressed significantly higher levels of CTSD, GRN and PSAP in an age-dependent manner, suggesting an up-regulation of the lysosomal network in disease. Upon siRNA KD in SHSY5Ys, we did not observe any interdependency of expression between the network members, but we are currently investigating whether genetic modulation of the network impacts GCase activity and whether the network is altered in GBA-PD mDA neurons.

Conclusions: In conclusion, our data support the need for further investigation of this lysosomal network in PD.
Aims: TFEB and TFE3 are master regulators of lysosomal biogenesis upregulating a gene network involved in lysosomal function, named the 'Co-ordinated Lysosomal Expression and Regulation' (CLEAR) network. Building evidence describes therapeutic potential of TFEB in PD models. Little work has been done to understand TFEB/TFE3 biology in a disease- and human-relevant model of PD. We aim to characterise TFEB/TFE3 biology, and their regulation upon lysosomal function in control- and patient-derived iPSC-derived dopaminergic neurons (iPSC-DAns).

Methods: Control and patient-derived iPSCs are differentiated into DAns using a common midbrain floor-plate progenitor protocol. TFEB/TFE3 expression and localisation, as well as CLEAR network regulation is assessed. Lysosomal biology and autophagic flux is assessed after TFEB/TFE3 modulation, using assays such as DQ-BSA, LysoPH, Lysosomal Calcium release, LAMP1 and LC3-flux, in both patient and control iPSC-DAns.

Results: TFE3 is preferentially expressed in iPSC-DAns and not TFEB. This is supported by analysis of published RNA-Seq datasets from both mouse and human brains. Neuronal maturation and modulation of TFE3-regulatory mechanisms can elevate TFE3 nuclear translocation, and CLEAR expression. Initial findings indicate altered TRPML1-evoked Ca\(^{2+}\)-release in patient-derived iPSC-DAns.

Conclusions: TFE3 is preferentially expressed in iPSC-DAns, not TFEB. This is recapitulated in publicly available human and mouse brain RNA-Seq datasets. TFE3 activation and CLEAR gene expression is upregulated as neurons mature, eluding to an increased demand upon the lysosomal system. Preliminary findings show altered lysosomal calcium release in iPSC-DAns derived from patients. Future work will probe novel TFE3 modulators and further observe how TFE3 modulation may be utilised to aid lysosomal/autophagy biology in iPSC-DAns.
POSTERS

DISSECTING THE ROLE OF GLUCOSYLCERAMIDE ACCUMULATION IN THE NEURONAL DAMAGE OCCURRING IN GBA-RELATED PATHOLOGIES

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Aims: β-glucocerebrosidase (GCase) is a lysosomal glycohydrolase encoded by GBA gene, responsible for the catabolism of the sphingolipid glucosylceramide (GlcCer). Deficiency of this enzyme causes the lysosomal accumulation of GlcCer, leading to the onset of GBA-related pathologies, characterized by neurological impairment and neurodegeneration, which comprise Gaucher Disease (GD) and GBA-dependent Parkinson’s disease (GBA-PD). Nevertheless, the relation between GCase loss of function and neurodegeneration is not understood so far.

Methods: To dissect the possible molecular mechanism linking GCase deficiency and the consequent GlcCer accumulation with the onset of neuronal damage occurring in GCase-related pathologies, we developed an in vitro human model of the neuronal form of GD represented by hiPSCs-derived dopaminergic neurons obtained from healthy subjects’ fibroblasts treated with 500 μM conduritol B epoxide (CBE), a specific GCase inhibitor.

Results: CBE-treated neurons present a progressive and time-dependent accumulation of GlcCer. Moreover, they recapitulate the neurodegenerative phenotype of GCase-related pathologies, presenting a significantly decreased expression of neuronal markers such as Tau, MAP2, Neurofilament H and PSD95. We also observed that GCase deficiency causes an enhanced lysosomal biogenesis and exocytosis, which leads to the extracellular release of uncatabolized GlcCer and to its accumulation also at the plasma membrane (PM) level.

Conclusions: These data let us to speculate about the existence of a lysosome-PM axis responsible for the alteration of the PM architecture that can leads to the neuronal damage occurring in GCase–related pathologies.
INVESTIGATING THE MOLECULAR MECHANISM LINKING B-GLUCOCEREBROSIDASE LOSS OF FUNCTION WITH NEURODEGENERATION IN GBA-PARKINSON’S DISEASE: PLASMA MEMBRANE AND METABOLIC IMPLICATIONS

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Aims: GBA-Parkinson’s Disease (PD) is caused by mutations in GBA gene, which are considered to be the major genetic risk factor for the development of PD, implying the deficiency of beta-glucocerebrosidase (GCase). GCase loss of function impairs the lysosomal catabolism of the sphingolipid glucosylceramide (GlcCer), causing its accumulation and the onset of neural damage. Nowadays, the implications of GCase loss of function on neurodegeneration are not completely clarified.

Methods: To this aim, we used as experimental model dopaminergic neurons derived from human iPSCs. After 30 days of treatment with 500 μM Conduritol B Epoxide (CBE), to suppress GCase activity, we performed: i) targeted metabolomics by LC-MS/MS; ii) isolation and characterization of plasma membrane (PM) detergent-resistant portions (DRM) obtained through ultracentrifugation on a sucrose gradient combined with biotin-streptavidin-mediated protein precipitation.

Results: We found that GlcCer accumulation changes the structure of the PM DRM. In particular, we identified the accumulation of GlcCer and the reduction of complex gangliosides together with an enrichment of the active form of c-Src. Regarding the metabolomics data, we discovered that the lysosomal impairment caused by GCase loss of function, is responsible for alterations in the overall cell metabolism and an increased uptake and use of aminoacids as energetic substrates.

Conclusions: The obtained data demonstrate that GCase loss function induces an impairment of the lysosomal compartment, that is responsible for two distinct events: i) the establishment of an aberrant lysosome-PM axis which alters the PM architecture with consequences on the intracellular signalling pathways; ii) alterations in the metabolic homeostasis of the neurons.
Aims: Introduction: Parkinson's disease (PD) is a neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra, which causes motor deficits. Accumulate evidence support that the chronic inflammatory response could mediate the neurodegeneration process. It’s been observed an increase in proinflammatory cytokines in the central and peripheral immune systems in PD patients. This increase in proinflammatory activity is induced by α-synuclein misfolded protein. Insulin-like growth factor 2 (IGF2), a possible mediator of the inflammatory in macrophages, is decreased in PD patients. We hypothesize that IGF2 deficiency promotes inflammation process mediated by TLR-4 in PD models. We evaluated the participation of IGF2 in the immune system modulation and their impact in neuroinflammation against the α-synuclein toxicity using in vitro and in vivo models of PD (ASO).

Methods: Materials & Methods: The inflammatory response was analyzed by flow cytometry, and PCR in macrophages isolated from ASO, treated with α-syn in the presence or absence of IGF2 in vitro. In ASO mice we evaluated the motor impairment (beam test, clapping test, and adhesive removal test), in presence of treated with macrophages+IGF2, as well as the presence of α-syn aggregates and inflammatory markers.

Results: Result: Here we observe a marked decrease of the inflammatory response in macrophages treated with α-syn in the presence of IGF2. in addition, we could observe a decrease of motor impairment, and proinflammatory markers in the ASO model treated with Macrophage+IGF2.

Conclusions: Conclusion: The treatment with Macrophage+IGF2 decreases chronic inflammation alleviating the motor impairment in in vivo model.
CHARACTERIZATION OF NK CELLS IN PARKINSON’S DISEASE

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Aims: Alpha-synuclein (α-syn) can self-assemble to form fibrillar aggregates and the accumulation of misfolded α-syn, a component of Lewy bodies, is the hallmark of PD pathology. Innate immune natural killer (NK) cells have been found to be increased in the blood of PD patients and to have decreased expression of the inhibitory receptor (NKG2A). We recently demonstrated that NK cells are present in the parenchyma of PD, are capable of clearing α-syn, and the depletion of NK cells resulted in exacerbated motor deficits and increased insoluble α-syn deposits in a preclinical mouse PD model.

Methods: To assess the role of NK cells in peripheral synuclein pathologies, NK cells were depleted in the preformed fibril α-syn-induced PD mice and we assessed α-syn pathology in the guts. To characterize NK phenotypes in PD. We performed flow analysis on cryopreserved PBMC from PD patients and measured the NK subsets (CD56 and CD16) and the phenotypic expression of inhibitory, activating, and homing receptors on their surface.

Results: Our data showed that peripheral synuclein pathologies in NK depleted mice were significantly exacerbated. We found that CD56dim NK subset is significantly increased and CD56- NK subset is trending decreased upon the disease severity (total UPDRS score) from populations grouped by relative CD56 (NCAM) and CD16 (FcγRIII) expression (CD56bright, CD56dim, CD56- NK subsets).

Conclusions: Our data implicate that the neuroprotective phenotype of NK cells is presented both in the CNS and periphery and highlight the potential capability of NK characterization as a potential biomarker for the early diagnosis of PD.
MUSCARINIC RECEPTOR EXPRESSION IN THE PERIPHERAL BLOOD CELLS DIFFERENTIATES DEMENTIA WITH LEWY BODIES FROM ALZHEIMER’S DISEASE


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Aims: Central Nervous System disruption of cholinergic (Ach) signaling, which plays a major role in cognitive processes, is well documented in Dementia with Lewy Bodies (DLB) and Alzheimer’s disease (AD). The expression of muscarinic Ach receptors type 1 and 4 (CHRM1 and CHRM4) has been reported to be altered in the brain of DLB patients. The aim of the study was to assess the peripheral gene expression of CHRM1 and 4 in DLB as a possible marker as compared to AD subjects and Healthy Controls (HC).

Methods: Peripheral mononuclear blood cells (PMBC) were collected from 21 DLB, 13 AD and 8 HC matched patients. Real time-polymerase chain reaction (RT-PCR) was performed to estimate the gene expression of CHRM1 and CHRM4.

Results: Peripheral CHRM1 expression was significantly higher in DLB and AD compared to HC, whereas CHRM1 and CHRM4 levels were higher in AD compared to DLB patients. Receiver operating characteristics curves, with logistic regression analysis, showed that combining peripheral CHRM1 and CHRM4 levels, DLB and AD subjects were classified with an accuracy of 0.76.

Conclusions: PBMCs gene expression of CHRM1 was higher and CHRM4 was lower in both DLB and AD patients than in HC. CHRM1 and CHRM4 gene expression resulted to be lower in DLB patients compared to AD. A less efficient peripheral compensatory mechanism of CHRM1 and CHRM4 gene activation is available in DLB. In the future, peripheral CHRM expression could be studied as a possible marker of neurodegenerative conditions associated with cholinergic deficit and a possible marker of response to AChEI.
A LONGITUDINAL EVALUATION OF THE PERIPHERAL IMMUNE PHENOTYPE IN A COHORT OF PARKINSON’S DISEASE PATIENTS

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Aims: To evaluate the suitable modifications of immunological parameters in a through characterized population of Italian PD patients

Methods: From 2014, drug naïve PD patients underwent a peripheral blood withdrawal annually, evaluating lymphocytes sub-populations and transcription factors (TF). Patients were excluded in presence of immune disease or immunomodulant/depressant treatment. Clinical and demographic parameters were monitored.

Results: 49 PD patients (33 male, mean age 68±8.4) with at least one follow-up visit were included. Th1 lymphocytes (as % of CD4+ cells) were higher after 2 and 4 years (V0: 15.91±6.61; V2:17.93±9.4; V4:20.88±11.6; p=0.03 and p=0.0006) while Th2 (as total count) were persistently reduced (V0:0.06*10³±0.02; V3: 0.04*10³±0.01; p=0.003). Th17 lymphocytes were reduced as percentage (V0: 8.13±4; V1:7.43±3.75; V4:7.84±0.84; p=0.04 and p=0.02) and total count (V0:0.07*10³±0.02; V1: 0.05*10³±0.03; V4: 0.05*10³±0.0; p=0.01 and p=0.01). Dealing with TF, STAT1 presented constantly increased levels (V0: 1.61*10^{-4}±0.0001; V1 2.39*10^{-4}±0.0001; V2: 2.38*10^{-4}±5*10^{-5}; V3: 2.86*10^{-4}±0.0001; respectively p=0.01; p=0.006; p<0.0001) while STAT6 levels were reduced (V0:6.96*10^{-6}±9.6*10^{-6}; V1: 9.01*10^{-7}±8.72*10^{-8}; V2: 1.51*10^{-6}±2.8*10^{-6}; p<0.0001 and p=0.0001). Total number of Treg was reduced in V3 and V4 (0.06*10³±0.02; V1: 0.05*10³±0.01; V4: 0.05*10³±0.01, p=0.008 and p=0.0004) and both activated and resting subsets. Accordingly, FOXP3 levels were significantly reduced at V4 compared to baseline (V0: 7.55*10^{-5}±6.4*10^{-5}; V4: 4.55*10^{-5}±5.01*10^{-5})

Conclusions: This is the first longitudinal study evaluating peripheral immune system in PD. Our data, though preliminary, indicate that the pro-inflammatory phenotype represents and early phenomenon in the disease decourse. Accordingly, immunotherapy in PD, which is under investigation, should be started soon in the disease history in order to act as disease modifier.
CHARACTERIZATION OF CHITINASE EXPRESSION IN SYNUCLEINOPATHIES

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\textbf{Aims:} Chitinases and Chitinase-Like proteins (C/CLPs) have recently been identified as key mediators associated with many inflammatory conditions including Alzheimer disease (AD) and amyotrophic lateral sclerosis (ALS), but no studies evaluating C/CLPs expression in synucleinopathies exist to date. Therefore, we aimed to characterize the expression patterns of Chitinase 1 (chit-1) and Chitinase 3-like protein 1 (CHI3L1) in animal models, as well as in human postmortem brain tissue of two synucleinopathies: Parkinson’s Disease (PD) and Multiple systems atrophy (MSA).

\textbf{Methods:} AAV was used to induce overexpression of αSyn in the rat brain to model synucleopathies. Specifically, AAV\textsubscript{9}-αSyn to target neurons of the substantia nigra and Olig001-αSyn to target oligodendrocytes of the striatum to model PD and MSA respectively. A combination of in situ hybridization (ISH) and immunohistochemistry was used to determine expression levels and cellular localization of Chit-1 and CHI3L1 in rat and postmortem human brain tissue sections.

\textbf{Results:} When compared to age matched neurological controls, significantly increased CHI3L1 expression was detected in cells with glial-like morphology in the substantia nigra (SN) of PD subjects. Likewise, increased CHI3L1-ir was detected in putamen of MSA subjects. Correspondingly, similar elevated expression of CHI3L1 was detected, specifically in astrocytes (GFAP+ cells), in both rat models of PD and MSA. Semi-quantitative assessment suggest Chit-1 is unchanged.

\textbf{Conclusions:} The current findings support the hypothesis that astrocytic CHI3L1 plays a role in synucleinopathies. Ongoing and future studies are aimed at modulating their expression to better understand its function and role in disease. Further, suggest a potential novel therapeutic target for neuroinflammatory diseases.
INFLUENCE OF GUT AND SYSTEMIC INFLAMMATION IN PARKINSON’S DISEASE USING A RECOMBINANT ALPHA-SYNUCLEIN BRAIN-INJECTION MODEL

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Aims: Parkinson’s disease (PD) patients often suffer from gut dysfunction accompanied by local inflammation, that can precede clinical diagnosis based on motor symptoms. This prodromal phase of PD gives rise to the possibility to investigate the interplay between peripheral inflammation and the brain pathology. More specifically, this project looks into the influence of systemic or gastrointestinal inflammation on the onset and progression of PD-like symptoms and the involvement of neuroinflammation in these pathological processes.

Methods: This by making use of an injection model of murine recombinant pre-formed fibrils (PFFs) of the hallmark PD protein, α-synuclein. PFFs were injected in the substantia nigra of wildtype mice. Next, a specific trigger was administered to induce systemic or gastrointestinal inflammation in this model. Motor function and behavior tests were performed on different timepoints following these injections to assess both motor symptoms and non-motor symptoms. The presence of α-synuclein aggregates was confirmed by phospho-α-synuclein staining of brain tissue. Neuroinflammation was assessed by microglia characterization and the loss of dopaminergic neurons was quantified.

Results: This model allows us to mimic the prodromal non-motor phase of PD, where we can study if a peripheral trigger, such as gut inflammation or systemic inflammation acts as an inducer or aggravator of the disease phenotypes and progression. By performing vagotomy (severing the vagal nerve) we will subsequently establish the specific role of the gut-brain axis in PD.

Conclusions: Through longitudinal studies, we characterize the appearance and development of both non-motor and motor symptoms and the involvement of the gut-brain axis in these pathological processes.
CHARACTERIZATION OF PERIPHERAL IMMUNE CELL STATE IN PARKINSON’S DISEASE

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Aims: A growing appreciation of the role of the immune system in Parkinson’s disease (PD) indicates that evaluation of immune cells may identify signatures relevant to the progression of the disease. We evaluated gene expression in peripheral immune cells at high resolution to identify PD-specific cell states.

Methods: We used single-cell transcriptomics (scRNA-Seq) to integrate and analyze data from over 17,000 peripheral blood mononuclear cells obtained from a cohort of PD patients and healthy volunteers.

Results: The study identified numerous genes differentially expressed in specific cell lineages in PD patients compared with healthy donors. In monocytes, 45 genes in the CD14 subset and 54 genes in the CD16 subset withstood FDR corrected statistical analysis. More robust changes were observed in the CD16 subset where increased expression of ATP5E, BRI3, PCBP, and FAM89B was consistently observable in PD patients compared with controls. While fewer CD16 monocytes were detected in PD overall, cells expressing high levels of TPGS1,SRM,YBX3, and MAP2K2 were clearly more numerous in PD patients compared with controls. We validated BRI3, a gene consistently upregulated in PD CD16 monocytes. In ongoing studies, CRISPR/CAS9 mediated inactivation of BRI3 altered the inflammatory response in cultured THP1 cells, a human monocytic cell line.

Conclusions: The study supports the existence of PD-specific immune cell states and identifies BRI3 as a novel immune modulator in PD. Data indicate alterations in the CD16 non-classical monocyte subpopulation, typically associated with the modulation of inflammatory processes, maintenance of vascular endothelial homeostasis, response to tissue injury, and chronic inflammatory disease.
THE EFFECTS OF ACTIVATED MICROGLIA ON THE PROPAGATION OF PATHOGENIC PROTEINS IN MOUSE MODELS FOR NEURODEGENERATIVE DISEASES

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Aims: Microglial activation has been implicated in the pathophysiology of various neurodegenerative diseases including Alzheimer’s and Parkinson’s disease. However, whether activated microglia could directly induce neurodegenerative features is yet to be known. In this study, we aim to investigate whether introduction of activated microglia into the striatum of naïve mice is sufficient to engender neurodegeneration.

Methods: SH-SY5Y neuroblastoma was treated with either α-synuclein or Tau proteins. Conditioned media collected from SH-SY5Y cells were treated to primary microglia derived from wild type C57BL/6 mice. After tagging with quantum dots (QDs) labeled with red fluorescence, microglial cells were injected into the unilateral striatum of naïve mice. Gliosis and proteinopathies were observed using immunohistochemistry (IHC), immunofluorescence (IF), and western blot (WB) assays. Several behavioral tests were used to evaluate motor and cognitive functions of mice.

Results: Data from IF assay demonstrated that primary microglia could successfully settle down in the striatum. QD-expressing microglial cells were strictly localized to the injected areas up to 3 months. Nonetheless, marked increases in microgliosis and astrogliosis were evident not only in the injected areas of striatum, but also in the other brain regions including motor cortex, rhinal cortex, and hippocampus.

Conclusions: Our data strongly suggested that localized delivery of activated microglia into the striatum is sufficient to induce gliosis over extensive areas of the brain. In our further studies, heterogeneity of microglia affected by either α-synuclein or Tau will be investigated by analyzing microglial subtypes and propagation of phosphorylated α-synuclein/Tau.
LRRK2-MUTANT MICROGLIA TRIGGERS DOPAMINERGIC NEURODEGENERATION WHEN ACTIVATED BY NEUROMELANIN

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Aims: Unravel pathogenic dysfunctional mechanisms due to LRRK2-G2019S mutation.

Methods: Use of human iPSC-based model to generate microglia (hMG) from LRRK2-PD and control iPSCs.

Results: We have generated microglia (hMG) from LRRK2-PD and control iPSCs and confirmed their identity by using specific microglial markers. We then carried out functional studies upon exposure to NM, which revealed a higher motility and phagocytic activity of LRRK2-PD hMG compared to control hMG. In addition, we found that extracellular NM particles induced microglial activation and increases ROS production in LRRK2-PD microglia. The use of a corrected isogenic PD hMG reverted all previous phenotypes, confirming a LRRK2-dependent activation of hMG. Upon co-culture with LRRK2-PD hMG and in the presence of NM particles, control vmDAns displayed morphological signs of neurodegeneration, such as short and few neurites as well as beaded necklace-like neurites, as well as increased neuronal loss.

Conclusions: Our findings indicate a critical role for neuromelanin-activated microglia in LRRK2-PD and may serve as a valid human cellular model to test compounds that can lower risk for PD or disease progression.
ASSESSING THE IMMUNOGENICITY OF α-SYNUCLEIN OLIGOMERS, FILAMENTS, AND FIBRILS IN A CLINICALLY RELEVANT MODEL OF SYNUCLEINOPATHY IN VITRO

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Aims: α-Synuclein (α-syn) is a protein implicated in the etiogenesis of synucleinopathic neurodegenerative disorders. Accumulation of α-syn spearheads the formation of Lewy bodies – small intracytoplasmic inclusions which lead to neuronal damage and death. Prior to Lewy Body formation and neurodegeneration, α-syn aggregates interact with glial cells, initiating inflammatory responses with consequences for neuronal health. We assess a putative role for glial cells as mediators of α-syn-induced neuronal atrophy, and specifically assess variant forms of α-syn in mediating these effects.

Methods: Primary rat mixed-glial cultures are treated with human recombinant wildtype α-syn preformed fibrils (PFFs), A53T mutant α-syn PFFs, soluble filaments, epigallocatechin gallate-stabalised oligomers, or dopamine-stabilised oligomers. Conditioned-media containing glial secreted inflammatory components is collected, filtered, and used to treat mature primary cortical neurons for 24 hours. Immunocytochemistry, qPCR, and multiplex infrared immunoassays are performed to determine changes in viability, cell morphology, activation state, and inflammatory profile in response to variant α-syn aggregates.

Results: Immunocytochemical analysis indicates no changes to primary microglial or astrocytic morphology, nor to neuronal complexity or synaptogenesis in response to wildtype α-syn PFFs. Further assessments are ongoing with oligomers and filaments. qPCR analysis of glial markers compliments cell phenotype associated with the application of various α-syn aggregates.

Conclusions: With the development of this model, cellular and molecular mechanisms underpinning the process by which α-syn mediates reactive-glial-associated neurodegeneration may be explored, and a platform upon which to develop neuroprotective treatment strategies targeting these mechanisms may be generated. Clinically, immunomodulatory agents may prove useful for treatment of neurodegenerative conditions associated with brain inflammation.
MICROGLIA-SPECIFIC KNOCK-OUT OF NF-KAPPAΒ/IKK2 INCREASES ALPHA-SYNucleIN PROTEIN AGGREGATION, GLIOSIS AND NEURODEGENERATION IN A MOUSE MODEL OF ROTENONE-INDUCED PARKINSON'S DISEASE

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Aims: Parkinson's Disease (PD) is the second most prevalent neurodegenerative disease worldwide, with limited treatments and no disease-modifying therapies. Progressive loss of dopamine neurons within the substantia nigra pars compacta (SNpc) results in neurodegeneration associated with neuroinflammation and misfolding of alpha-synuclein. Despite intensive research efforts, the etiology and progression of pathology remain poorly understood. Recently, environmental toxicants that inhibit mitochondrial respiration at complex I, such as the pesticide rotenone, have been implicated as potential risk factors for PD. Rotenone, like other pesticides causing mitochondrial dysfunction, are potent activators of innate inflammatory signaling pathways in microglia that cause neuroinflammation associated with disease progression. To understand the role of inflammatory activation of microglia in rotenone-induced neurodegeneration, we therefore sought to describe temporal and regional changes in patterns of glial activation, neuronal injury and alpha-synuclein protein aggregation in mice systemically exposed to rotenone. We postulated that loss of NFkB signaling in microglia would attenuate rotenone-induced inflammatory injury to the nigro-striatal dopamine system and prevent aggregation of alpha-synuclein.

Methods: This work was conducted in wildtype C57Bl/6 mice and in novel microglia-specific knockouts for the inflammatory transcriptional factor, NF-kappaB (NFkB).

Results: Multi-regional montaging analysis of alpha-synuclein misfolding and accumulation, gliosis, and neurodegeneration revealed PD progression in region and time-specific manners where microglial NFkB knockout surprisingly led to increases in neurodegeneration, protein aggregation within microglial cells, and multi-faceted glial reactivity.

Conclusions: These results confer that microglial mediated inflammatory responses are necessary within neurodegeneration to aid in the clearance of protein aggregates and without these processes, excess protein aggregation further exacerbates neurodegeneration.
INVESTIGATING THE ROLE OF MICROGLIA IN ALPHA-SYNUCLEIN PATHOLOGY IN A HUMAN IPSC-DERIVED CO-CULTURE MODEL

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Aims: Alpha-synuclein (aS) aggregation is one of the hallmarks of Parkinson’s disease (PD) and evidence is accumulating that microglia (MG) play an important role in this process. However, much of what we know about this comes from rodent or 2D cell culture experiments which may not fully represent the human brain. Hence, the objective of this study was to investigate the interaction of aS aggregation and MG in a human 3D cell culture model.

Methods: We used previously published protocols (Yoon et al., 2019; Haenseler et al., 2017) to generate human induced pluripotent stem cell (iPSC)-derived cortical spheroids (hCS) and MG. Pre-formed fibrils (PFFs) derived from recombinant aS were used to induce aggregation in the cultures.

Results: iPSC-derived hCS reproducibly contained superficial and deep layer cortical neurons as well as astrocytes. MG expressed typical markers and displayed characteristic ramified morphology and phagocytic activity in monoculture. We established a protocol to create co-cultures between iPSC-derived MG and hCSs. MG precursor cells readily migrated into the spheroids and retained morphological and functional MG characteristics. Seeding with PFFs induced PS129 positive inclusions in hCSs.

Conclusions: Here we present the ‘next generation’ of PD modelling. Due to its human origin, 3D structure and the presence of microglia in addition to neurons, astrocytes and oligodendrocytes, we believe this model closely recapitulates the human disease. This co-culture model is a powerful tool to investigate the molecular processes underlying synucleinopathies and identify relevant therapeutic targets.
POSTERS

NCX3-INDUCED MITOCHONDRIAL DYSFUNCTION IN MIDBRAIN LEADS TO NEUROINFLAMMATION IN STRIATUM OF A53T-ALPHA-SYNUCLEIN TRANSGENIC OLD MICE

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Aims: The complex interplay among toxic alpha-synuclein aggregates, oxidative stress, altered intracellular Ca^{2+}-homeostasis, mitochondrial dysfunction and disruption of mitochondrial integrity is considered among the pathogenic mechanisms leading to dopaminergic neuronal loss. Herein the molecular mechanisms leading to mitochondrial dysfunction and their relationship with activation of the neuroinflammatory process occurring in Parkinson’s Disease have been investigated.

Methods: Experiments were performed in vitro and in vivo in mice carrying the human mutation of α-synuclein A53T under the prion murine promoter. In these models, the expression and activity of NCX isoforms, a family of important transporters regulating ionic homeostasis in mammalian cells working in a bidirectional way, were evaluated in neurons and glial cells. Mitochondrial function was monitored with confocal microscopy and fluorescent dyes to measure mitochondrial calcium content and mitochondrial membrane potential. Parallel biochemical analysis was performed in 4 and 16 months old A53T-alpha-synuclein-Tg-mice to correlate the functional data obtained in vitro with mitochondrial dysfunction and neuroinflammation.

Results: The results demonstrated: 1. in A53T-alpha-syn-mice mitochondrial dysfunction occurs early in midbrain and later in striatum, 2. mitochondrial dysfunction occurring in the midbrain is mediated by NCX3 protein expression impairment in neurons and astrocytes, 3. mitochondrial dysfunction occurring early in midbrain triggers neuroinflammation later into striatum, thus contributing to PD progression during mice aging.

Conclusions: Mitochondrial dysfunction occurring in A53T-alpha-syn-mesencephalic-neurons at the early stage of the disease promotes neuronal degeneration and activates microglial cells in the striatum where, promoting pro-inflammatory factors release and glial activation, causes impairment of dopaminergic neuronal plasticity in the late stage of the disease.
**UBA52 PREVENTS OXIDATIVE STRESS AND MITOCHONDRIAL DYSFUNCTION THROUGH VDAC1 UBIQUITYLATION: IMPLICATIONS IN PARKINSON’S DISEASE**

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**Aims:** Protein aggregation is considered as major pathological hallmark of Parkinson’s disease (PD) due to presence of lewy bodies in post-mortem brain of PD, suggesting impaired energy and mitochondrial dysfunction that require protein degradation pathways like ubiquitin proteasome system (UPS). Here, we have shown the correlation of mitochondrial function with principal component of UPS, ubiquitin encoded by UBA52 during diseased conditions.

**Methods:** Estimations were done in both cellular and sporadic rat model of PD. The expression level of UBA52 in diseased conditions was assessed and further implications of UBA52 in disease related neurodegenerative signalling were investigated employing overexpression strategies.

**Results:** Significant downregulation of UBA52 was observed during disease in both experimental models. Further, the transient expression of Myc-UBA52^{+/+} in neuronal cells altered disease related ROS generation, increased nitrite level and depletion in glutathione level. UBA52 overexpression also improved mitochondrial functionality by preventing change in mitochondrial membrane potential, mitochondrial complex-I and cell viability, intracellular calcium uptake and lessened mitochondrial permeability transition pore opening (cytochrome-c release). Mass spectrometric data and co-immunoprecipitation studies suggested the interaction of UBA52 with mitochondrial outer membrane channel protein, VDAC1 in both neuronal cells and dopaminergic region of rat brain. We found that UBA52 attached lysine-48 linked ubiquitin chain to VDAC1 in E3 ubiquitin ligase CHIP mediated ubiquitylation. Additionally, Myc-UBA52^{+/+} expression protected dopaminergic neurons against apoptotic (Bcl-1, Bax) and autophagic (p62, Beclin1 and LC3) cell death.

**Conclusions:** Our findings delineate correlation between UBA52 and mitochondrial homeostasis, providing new insights into the dopaminergic cell death during PD pathogenesis.
INVESTIGATING THE CELLULAR AND MOLECULAR RESPONSE OF HUMAN DOPAMINERGIC NEURONS TO MITOCHONDRIAL STRESS

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Aims: In this study, we aim to characterise unbiasedly the mitochondrial stress response of human DA neurons at the transcriptome level. To discover specific molecular mechanisms underlying DA neurons’ enhanced vulnerability to mitochondrial stress, we include analysis of highly cell-specific genomic non-coding elements, such as long non-coding RNAs (lncRNAs) and open regions of the chromatin (ORCs).

Methods: Using RNA-seq and ATAC-seq, we generated transcriptomic and chromatin accessibility data from Lund Human MESencephalic cells-derived DA neurons, in control or mitochondrial stress conditions. Using RT-qPCR, immunofluorescence and Western Blot, we confirmed the activation of several pathways in response to stress.

Results: We demonstrated for the first time concomitant activation of several endoplasmic reticulum Unfolded Protein Response (UPR) pathways in human DA neurons upon mitochondrial stress, leading to engagement in apoptotic signaling. Importantly, we identified lncRNAs specifically expressed (19%) or inhibited (33%) following stress. Analysis of these lncRNAs and putative gene targets suggests their contribution to specific steps of DA neurons’ stress response, such as regulation of translation, mediated by the mTOR pathway, which is altered in PD. Cross-analyses with public ChIP-seq datasets, reveals potential transcriptional regulation of 47% stress-associated lncRNAs by TRIM24, actor of the mTOR pathway. TRIM24 is also associated to 28% of stress-linked ORCs, including 41 out of 71 containing PD-associated SNPs.

Conclusions: This work provides invaluable knowledge to 1) further understand lncRNAs’ role in the DA stress response and 2) identify critical elements of the DA neuronal stress response that are altered in PD.
STUDYING MITOPHAGY IN NEURONAL MODELS OF ALPHA-SYNUCLEINOPATHY WITH THE FLUORESCENT MITOROSELLA REPORTER

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Aims: The aim of this study is to explore the impact of the abnormal accumulation of alpha-synuclein (aSyn) on mitochondrial quality control mechanisms in neurons, with a focus on mitophagy.

Methods: We used a model of primary mouse cortical neurons (E16) in which aSyn aggregation is promoted by fibrils of normal human aSyn (f91) preformed in vitro. Experiments were performed in both wild type type neurons and neurons deficient for Parkin, to explore specifically the PINK1/Parkin-dependent mitophagy pathway. Mitophagy was investigated in live neurons with the fluorescent MitoRosella reporter, monitoring the presence of mitochondria within lysosomes. The reporter was expressed by means of lentiviral vector-mediated gene delivery.

Results: At two weeks of treatment with f91, 30% of the neuronal cell bodies and many neuronal processes contained deposits of phosphorylated aSyn (P-aSyn). Volumetric co-localization analysis revealed a more than 40% overlap between mitochondria stained for TOM20 and P-aSyn immunoreactive deposits. Synucleinopathy was associated with a 60-100% increase in the Mito-Rosella fluorescent signal associated with lysosomes, in both wild type and Parkin-deficient neurons. This increase was partially reversed by treatment with 3-methyladenine (3-MA) or bafilomycin A1 (4h). By contrast, exposure of neurons to the mitochondrial complex III inhibitor antimycin A (100 nM, 3h) enhanced mitophagy by 150% in wild-type neurons only, and this effect was completely reversed by 3-MA.

Conclusions: Synucleinopathy mediated by f91 stimulates the autophagy of mitochondria in a PINK1/Parkin-independent manner. Further studies are required to evaluate possible effects of aSyn accumulation on non-elective bulk autophagy and lysosomal function.
ENDURANCE EXERCISE CONTRIBUTES TO NEUROPROTECTION AGAINST A MPTP-INDUCED PARKINSON-LIKE SYMPTOMS IN MICE VIA MODULATION OF MITOCHONDRIAL PHENOTYPES

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Aims: Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by loss of dopaminergic neurons in the substantia nigra, leading to motor deficit. Endurance exercise (EE) has been known to exert neuroprotective effects against PD. However, the molecular mechanisms underlying the protection have not been fully elucidated.

Methods: In this study, we investigated whether EE-induced neuroprotection is associated with mitochondrial phenotypes using a 1-methyl-1,2,3,6-tetrahydropyridine with probenecid (MPTP/P)-induced mouse model of PD. Seven weeks old C57BL/6 male mice were randomly assigned to three groups: control (CON), MPTP/P (MPTP/P) and MPTP/P plus endurance exercise (MPTP/P +EE). Mice assigned to endurance exercise performed treadmill running at 12 m/min for 60 min/day, 5 days/week for 8 weeks.

Results: Our data showed that EE intervention ameliorates MPTP/P-induced motor dysfunction in parallel with reduced dopaminergic neuronal cell death. More importantly, EE significantly enhances mitochondrial phenotypic changes such as upregulated mitochondrial biogenesis (e.g., PGC1α and Tfam), fusion (e.g., OPA1 and MFN2), and mitophagy (e.g., PINK1, PARKIN and LC3).

Conclusions: Taken together, our data suggested that EE-induced mitochondrial phenotypic changes are linked to neuroprotection against MPTP/P-induced neurotoxicity.
Aims: (Poly)phenols-enriched diets have been associated to brain’s health with positive impact towards still cureless neurodegenerative disorders, as Parkinson’s Disease (PD). After ingestion, (poly)phenol-rich foods originate low-molecular weight (poly)phenol metabolites (LMWPM) found in circulation that potentially reach the brain, which may constitute true effectors against cellular and molecular mechanisms of PD.

Methods: The present study investigates the neuroprotective potential of two LMWPM metabolites, catechol-sulfate and pyrogallol-sulfate, in a 3D human cell model of PD, generated from LUHMES cell line challenged by 1-methyl-4-phenylpyridinium (MPP+).

Results: LMWPM were differently neuroprotective towards MPP+ insult. From an integrated transcriptomic analysis, both metabolites differently modulated gene expression, suggesting that, prior to the dopaminergic insult, the neuronal cells presented unequal starting points. In particular, catechol-sulfate increased the expression of genes associated to oxidative stress response, suggesting a hormetic mode of action. Moreover, when MPP+ is applied, LMWPM pre-treatment positively modulated glutathione metabolism, apoptotic proteins balance as well as heat-shock response.

Conclusions: Our findings point, for the first time, to circulating LMWPM potential in triggering molecular mechanisms to help dopaminergic neurons to cope with a later and stronger insult, comprising promising molecules to be further explored in the scope of PD.
DOPAMINERGIC NEURONS GENERATED FROM IPSCS CARRYING MUTATIONS IN EITHER SNCA, GBA OR LRRK2 RECAPITULATE SALIENT PARKINSON’S DISEASE PHENOTYPES

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Aims: Human iPSC-derived dopaminergic neurons offer a developmentally and physiologically relevant in-vitro model of human midbrain dopaminergic neurons that innervate different regions in the CNS including the forebrain and striatum. Loss of dopaminergic neurons cause decreased dopamine levels in the CNS and result in neurodegenerative conditions including Parkinson’s disease (PD). Dopaminergic neurons decline with age and are selectively vulnerable to oxidative stress generated by dopamine oxidation that increases with age.

Methods: The present study outlines the generation and characterization of iPSC derived dopaminergic neurons from two PD patients that inherited either the GBA (N370S) or LRRK2 (G2019S) mutation (GBA and LRRK2 lines are part of the Parkinson’s Progression Markers Initiative (PPMI) iPS cell bank) and genetically engineered SNCA A53T iPSCs.

Results: End stage dopaminergic neurons derived from healthy as well PD donors were assessed for GBA activity, neuronal MEA activity, and alpha-synuclein-mediated protein aggregation. These results recapitulated the many features of PD in a dish.

Conclusions: This panel of human iPSC-derived dopaminergic neurons carrying disease-specific mutations can be used for various in vitro applications to uncover mechanistic insights of dopaminergic neuronal degeneration and identification of novel therapeutic targets.
Aims: The aim of this project is to identify disease-associated cellular phenotypes in Miro1 mutant models and to find novel targets to correct impaired Miro1 function in Parkinson’s disease (PD).

Methods: We analyzed Miro1 removal from damaged mitochondria in native skin fibroblasts from PD patients carrying the Miro1 R272Q and R450C mutations. Mitochondrial bioenergetics in iPSC-derived neurons from a PD patient carrying the R272Q mutation was measured with the Seahorse technology. We generated R285Q mice (R272Q orthologue) to assess the mutation’s impact on nigrostriatal pathway’s integrity, by staining the striatum and substantia nigra pars compacta (SNpc) for tyrosine hydroxylase and dopamine transporter, and measured striatal dopamine levels by gas chromatography.

Results: In native fibroblasts, the R450C mutant degrades Miro1 upon mitochondrial depolarization, while this is less evident in the R272Q mutant. In iPSC-derived neurons, the R272Q Miro1 mutation displays a significantly decreased oxygen consumption rate, highlighting its impact on energy metabolism. In vivo, the R285Q mutation affects neither the striatum’s structural integrity nor neuronal viability in the SNpc of young and aged mice. Striatal dopamine levels in aged Miro1 R285Q-mutant mice are unaffected.

Conclusions: The increased stabilization of Miro1 in R272Q mutant fibroblasts could bring the project to a translational phase, by testing a “Miro1 reducer” compound in R272Q mutant neurons and fibroblasts to correct pathological phenotypes. We highlighted that the R272Q mutation causes alterations in energy metabolism of iPSC-derived neurons in vitro. In vivo, aging is not sufficient to induce dopaminergic neurodegeneration in mice expressing the human orthologue R285Q.
POSTERS

QEEG-BASED DIFFERENTIATION BETWEEN ALZHEIMER’S DISEASE WITH OR WITHOUT ALPHA SYNUCLEINOPATHY

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Aims: As existence of alpha synucleinopathy could affect progression of cognitive impairment in Alzheimer’s disease (AD), it’s very important to differentiate comorbidity of alpha synucleinopathy in AD to predict progression. To develop affordable EEG-based discriminating machine-learning(ML) algorithm for existence of alpha synucleinopathy, we explore differences between AD with vs without it.

Methods: Based on pattern of cognitive impairment and 3 types of PET scan [18F-Florbetaben brain amyloid-beta, FDG, DAT-PET], dementia due to Alzheimer’s disease [pure ADD] or Lewy Body (pure LBD) or mixed type (AD with LB disease) were clinically classified. We measured 19ch resting state EEG based on international 10-20. Quantitative analysis of EEG was done by iSyncBrain®.

Results: Pure ADD showed the characteristics that general EEG slowing with low total power, relative delta enhancement and desynchronized alpha with low amplitude. Pure LBD showed the pattern of slow alpha peak frequency with intact synchronization, relatively higher EEG total power and frontotemporal theta enhancement. Compared to pure ADD, ADD with LB showed mixed pattern of ADD and LDB. Total power was higher, alpha wave showed intact synchronization but theta power was rather enhanced at frontal(Fz; p-value < 0.05) and bilateral temporal(T3,5, T4,6; p-value < 0.05) area that was reported as characteristic pattern of progressive type of AD by previous studies.

Conclusions: These findings imply that ADD could have different EEG oscillation characteristics.
according to the Lewybody disease. Next step, we will develop the machine-learning algorithm to discriminate the Lewybody disease based on specific EEG features and validate it.
Aims: Most neurodegenerative disorders predominantly target the synapses. Indeed, both amyloid precursor protein and alpha synuclein are found in the synapses abundantly and believed to be involved in synaptic (dys)function. Thus, it is essential to study aggregate accumulation and their role in synaptic loss, to understand degeneration mechanisms and develop treatments. The aim of this study is to develop tools to characterise synaptsomes from dementia models, using super-resolution microscopy.

Methods: Synaptosomes are prepared by physically detaching the axon terminal from the rest of the neuron by centrifugation. A membrane bubble is formed as the loose ends fuse. Synaptosomes contain pre-synaptic scaffolding and SNARE proteins, as well as neurotransmitter vesicles. Here, we prepared synaptosomes from iPSC neuronal models of Parkinson's disease with a SNCA gene triplication. Single molecule pulldown (SiMPull) was used to attach the synaptosomes to PEG coated glass coverslips, using biotinylated Neurexin1 antibodies to capture them. Upon fixation and permeabilization, alpha synuclein aggregates inside the synaptosomes were quantified by diffraction-limited fluorescence microscopy and characterised in terms of their size and shape using stochastic optical reconstruction (STORM) microscopy. This high-throughput super-resolution technique relies on photobleaching fluorophores with high laser power and randomly reactivating them using a buffer -providing post diffraction-limited resolution of 20 nanometres.

Results: By harvesting the neurons at different time points, we were able to study disease progression and alpha synuclein accumulation, including the size and shape distribution of the aggregates.

Conclusions: By combining SiMPull and STORM, we were able to characterise the alpha synuclein aggregates in synaptosomes for the first time.
INVESTIGATING MECHANISMS OF NEUROPLASTICITY IN A HUMAN MIDBRAIN ORGANOID MODEL OF PARKINSON'S DISEASE

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Aims: To investigate whether impaired neuroplasticity precedes neurodegeneration in midbrain organoids derived from Parkinson's disease (PD) patients, and whether the neuroplasticity deficits contribute to the eventual loss of dopaminergic neurons in PD.

Methods: Midbrain organoids are generated from two PD patient induced pluripotent stem cell lines with the SNCA triplication mutation, along with two sex-age matched healthy control lines to investigate phenotypes related to neuronal plasticity. We investigate differences between wild type and SNCA mutant organoids with respect to neural stem cell activity as well as neuronal, synaptic and astrocytic development at 15, 21, 30, 35, 70 and 90 days of organoid maturation. This is done by assessing the expression of relevant key markers using immunofluorescence stainings and Western blots at each time point.

Results: Organoids derived from PD patients with the SNCA triplication mutation show increased stem cell activity and accelerated neuronal differentiation in comparison to healthy controls up until 35 days of organoid maturation. At 70 and 90 days, the amount of dopaminergic neurons are lower in the mutants compared to the controls, possibly indicative of degeneration in the mutants. We also observe nuclear laminar deficits in the cells within the mutant organoids, as well as downregulation of synaptic proteins such as VAMP2.

Conclusions: SNCA triplication organoids show an atypical development, which may be related to altered neuronal plasticity caused by the mutation, increasing vulnerability to degeneration.
HUMAN ALPHA-SYNUCLEIN OVEREXPRESSION IN MOUSE SEROTONIN NEURONS ELICITS A DEPRESSIVE-LIKE PHENOTYPE: FOCUS ON BRAIN CONNECTIVITY AND SYNAPTIC DENSITY


Aims: Besides the motor symptoms that define Parkinson's disease (PD), up to 50% of patients experience cognitive decline and psychiatric disorders, among which anxiety and depression are the most prevalent neuropsychiatric symptoms. Although dopamine system deficits are involved in several non-motor manifestations, structural and functional alterations in the serotonin (5-HT) system also occur in PD and may contribute to non-motor phenotypes. In this study, we investigated how α-synucleinopathy in the 5-HT system triggers synaptic alterations in the brain circuits involved in emotional and mood control.

Methods: We used a new mouse model of α-synucleinopathy in the 5-HT system, based on AAV5-induced overexpression of wild-type human-α-synuclein (h-α-Syn) in raphe nuclei. Mice were assessed at 4 and 8 weeks later. Cytoskeletal components and synaptic vesicle SV-associated proteins were examined by confocal microscopy. Brain functional connectivity was analyzed in the resting state (rsfMRI) by BOLD signal. The cellular activity was measured by Egr-1 mRNA expression in different brain regions.

Results: Overexpression of h-α-Syn in 5-HT neurons leads to progressive reductions of MAP-2 density in different projection brain regions, including prefrontal, cingulate and motor cortices and caudate-putamen. Simultaneously, h-α-Syn mice also showed changes in SV2A and synaptophysin levels in some of the analyzed brain regions. Hypoconnectivity in caudate-putamen and hippocampus, as well as increased Egr-1 mRNA expression, were detected 8-weeks later.

Conclusions: These data indicate that presynaptic h-α-Syn accumulation in 5-HT neurons causes alterations in crucial components for the synaptic structure and function in circuits involved in emotional and mood control in PD.
SYNAPSE-SPECIFIC REGULATION OF GLUTAMATE RELEASE BY α-SYNUCLEIN IN THE BASOLATERAL AMYGDALA

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Aims: α-Synuclein (αSyn) localizes preferentially at presynaptic boutons, where it is thought to regulate synaptic transmission and plasticity. Recent studies show that αSyn is also preferentially expressed in specific cell types in different brain regions. Yet, it remains unclear if different subtypes of synapses contribute to this pattern of expression.

Methods: We employed immunohistochemistry, electrophysiology and confocal microscopy to study the distribution of αSyn at glutamatergic presynaptic terminals in the basolateral amygdala (BLA).

Results: Using immunohistochemical approaches, we revealed that under physiological conditions αSyn is preferentially found in the glutamatergic axon terminals arising from the cerebral cortices. Interestingly, axon terminals arising from subcortical regions were practically devoid of αSyn. We then determined whether this preferential localization is important for synaptic function using ex vivo electrophysiology. We found that in mice lacking αSyn, the cortical glutamatergic synaptic transmission was impaired in response to continuous stimulation while the transmission between the thalamus and the BLA was not affected. Moreover, using an αSyn preformed fibril model, we showed that the formation of pathological αSyn inclusions significantly diminishes the amount of αSyn in the cortical axon terminals, and selectively disrupts glutamatergic synaptic transmission between the cerebral cortex and the BLA. Our observations indicate that a reduction in the amount of αSyn available in the synapse, likely caused by the sequestration of soluble αSyn into aggregates, has functional consequences.

Conclusions: In conclusion, we have compelling evidence indicating that the loss of normal function of αSyn is a key mechanism underlying the disrupted synaptic transmission caused by the formation of αSyn inclusions.
Aims: Parkinson's disease (PD) is an age-related neurodegenerative disease that has sporadic or genetic origin. It is characterized by the loss of dopaminergic neurons and presence of cytosolic inclusions named Lewy Body. Genetic evidences indicate that two proteins, namely parkin and alpha-synuclein are key contributors to PD. Parkin displays both transcription factor and E3 ubiquitin-ligase functions. Mutations of parkin (PRKN) gene are associated with autosomal inheritance. Alpha-synuclein is a protein involved in several neuronal processes, as neuronal plasticity and regulation of neurotransmitters. It is a central protein in the physiopathology of PD and a major component of Lewy Body. The alpha-synuclein is degraded by chaperone mediated autophagy (CMA) and its accumulation is associated with neuronal death in PD. Our objective was to establish whether a functional link occurs between alpha-synuclein and parkin and if it is altered in PD.

Methods: We verified alpha-synuclein regulation by parkin through biochemical approaches. We used several overexpressed and depleted parkin cellular models, parkin invalidated brain mice and PD human brain samples.

Results: We demonstrate that overexpression and depletion of parkin inversely regulate alpha-synuclein protein and mRNA levels in vitro. Interestingly, the knockout of parkin modulates alpha-synuclein protein and mRNA levels differently according with age of mice brain. Moreover, in sporadic PD human brain samples we have observed a correlation between alpha-synuclein and parkin protein levels.

Conclusions: Alpha-synuclein is regulated by parkin protein ex-vivo and in vivo.
Aims: Several missense mutations of the SNCA gene such as A53T and A30P as well as multiplications of the SNCA gene locus have been linked to familial forms of Parkinson’s disease and parkinsonism-dementia syndrome. Pathogenesis of these SNCA variants is different, but they all lead to accumulation of alpha-synuclein aggregates. To better understand these pathogenic mechanisms, we aimed to identify transcriptome and translatome of each variants in dopaminergic cells and define specific or common disturbances.

Methods: We generated SH-SY5Y cell lines, stably overexpressing the SNCA gene (SNCA 140, SNCA 112 and SNCA A53T) as well as controls by lentiviral transduction. Then, we started the study of small neuronal precursor cells (smNPC cells) derived from PD patient fibroblasts with an SNCA triplication and A30P mutation as well as controls. We performed RT-qPCR and Western-blot to study expression of transcripts and proteins involved in different steps of the translation process. Polysome profiling analyses are also ongoing to define the global translation of the cells.

Results: Preliminary data in SH-SY5Y cells suggest the absence of strong differences between polysomes profiles of SNCA isoforms or pathogenic variants versus control conditions. However, the ongoing study of qPCR and phosphorylated/total ratio of several initiation or elongation factors suggests transcription and/or translation deregulations linked to some of these models.

Conclusions: The first observations sustain the existence of transcription and translation perturbations in these models and their characterization will pursue.
A COMPARISON OF THE LEVELS OF ALPHA-SYNUCLEIN IN PATIENTS WITH LATE-ONSET SCHIZOPHRENIA, PARKINSON'S DISEASE AND A CONTROL GROUP

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Aims: To evaluate differences between levels of alpha-synuclein in patients with late-onset schizophrenia, Parkinson's Disease, and controls

Methods: We estimated alpha-synuclein level in a homogeneous purified lymphocytic cell fraction using magnetic sorting with antibodies specific to the CD45+ cell receptor conjugated on them from patients with Parkinson’s disease (PD) N=114, patients with late-onset schizophrenia (LOS) N=42, and healthy individuals (control) N=104. The level of alpha-synuclein in CD45+ cells was estimated by enzyme immunoassay (ELISA) (Human alpha-synuclein ELISA kit (Invitrogen, USA)) using a BioRadxMark microplate spectrophotometer (USA). Each sample was measured in triplicate. The normalization was carried out for the total protein. The study was approved by the local ethics committees. All the participating subjects provided informed consent. To assess differences between groups, the Mann–Whitney test was used, and the correlations were evaluated by exponential regression analysis. Experimental data are given as median (min–max). The level of significance was set at p<0.05. Statistical analysis was carried out using SPSS 12.0

Results: An increase alpha-synuclein level was shown in CD45+ cells of patients with LOS (9.21 (0.78-29.52) median (min–max)) compared to control (6,355 (0.46-35.44)) (p=0.024). At the same time, there were no significant differences in alpha-synuclein level of patients with PD (7.24 (0.46-40.28)) compared to patients with LOS and control.

Conclusions: The neurobiological aspects of LOS remain unknown, detected increase in the level of alpha-synuclein probably suggests previously unknown mechanisms of pathogenesis of LOS (as synucleinopathy?), which requires further study including the mRNA SNCA in patients with LOS and PD and conceptualization of the obtained data.
POSTERS

CRYO-EM STRUCTURE OF ALPHA-SYNUCLEIN FIBRILS AMPLIFIED BY PMCA FROM PD AND MSA PATIENT BRAINS

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Aims: Synucleinopathies are neurodegenerative diseases related to the aggregation of the protein alphasynuclein (aSyn). Among these diseases, Parkinson’s disease (PD) and multiple system atrophy (MSA) are most prevalent. aSyn can readily form different fibrillar polymorphs, if exposed to an air-water interface or by templating with pre-existing fibrils. Here we aim to determine the structure of aSyn filaments derived from human brain homogenates.

Methods: Seeding monomeric aSyn with PD and MSA patients brain homogenates using protein misfolding cyclic amplification (PMCA) and subsequential cryo-EM of fibrils followed by helical reconstruction

Results: The amplified fibrils reveal new structures from PD and MSA brain tissue, which partly resemble that of previously reported in vitro generated fibrils from Y39 phosphorylated aSyn protein.

Conclusions: The fibril structures reported here were obtained after seeding from diseased human brain homogenate and differ from all previously published aSyn fibril arrangements. In case these fibrils would turn out to be the long sought causative agents of these diseases, their structures might lead to the development of therapeutic strategies to modify these diseases and to a better understanding of the mechanistic processes that lead to neurodegeneration and spreading of the diseases. The relevance of these fibrils for synucleinopathies in humans remains to be further investigated.
A COMPARISON OF ALPHA-SYNUCLEIN LEVELS IN PATIENTS WITH LATE-ONSET SCHIZOPHRENIA, PARKINSON'S DISEASE, AND CONTROLS.

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Aims: To evaluate differences between levels of alpha-synuclein in patients with late-onset schizophrenia, Parkinson's disease, and controls.

Methods: We estimated alpha-synuclein level in a homogeneous purified lymphocytic cell fraction using magnetic sorting with antibodies specific to the CD45+ cell receptor conjugated on them from patients with Parkinson's disease (PD) N=114, patients with late-onset schizophrenia (LOS) N=42, and healthy individuals (control) N=104. The level of alpha-synuclein in CD45+ cells was estimated by enzyme immunoassay (ELISA) (Human alpha-synuclein ELISA kit (Invitrogen, USA)) using a BioRadxMark microplate spectrophotometer (USA). Each sample was measured in triplicate. The normalization was carried out for the total protein. The study was approved by the local ethics committees. All the participating subjects provided informed consent. To assess differences between groups, the Mann–Whitney test was used, and the correlations were evaluated by exponential regression analysis. Experimental data are given as median (min–max). The level of significance was set at p<0.05. Statistical analysis was carried out using SPSS 12.0

Results: An increase alpha-synuclein level was shown in CD45+ cells of patients with LOS (9.21 (0.78-29.52) median (min–max)) compared to control (6.355 (0.46-35.44)) (p=0.024). At the same time, there were no significant differences in alpha-synuclein level of patients with PD (7.24( 0.46-40.28)) compared to patients with LOS and control.

Conclusions: The etiology of LOS remains unknown, the detected increase in the level of alpha-synuclein probably suggests previously unknown mechanisms of pathogenesis of LOS (as a synucleinopathy?), which requires further study including the mRNA SNCA in patients with LOS and PD and conceptualization of the obtained data.
ALTERATIONS IN SELF-AGGREGATING NEUROPEPTIDES IN CSF OF PATIENTS WITH PARKINSONIAN DISORDERS

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Aims: Development of therapies for neurodegenerative diseases can be improved with a better understanding of molecular deficits and etiologies. PD, PSP and MSA present with similar movement disorder symptoms but distinct protein aggregates upon pathological examination. We seek candidate biomarkers in parkinsonian disorders for differential diagnosis of subgroup molecular etiologies.

Methods: Untargeted liquid chromatography (LC)-mass spectrometry (MS) proteomics was used for discovery profiling in CSF followed by LC-MS/MS based multiple reaction monitoring (MRM) for validation of candidates. We used both univariate analysis and multivariate statistical modeling to determine changes in protein levels for each disease compared to healthy controls. We compared clinical variation within the parkinsonian cohort including PD subgroups exhibiting tremor dominance (TD) or postural instability gait disturbance (PIGD) and those with detectable leukocytes in CSF.

Results: We have identified candidate peptide biomarkers and have validated related proteins with targeted quantitative multiplexed assays. Dopamine-drug naïve patients at first diagnosis exhibit reduced levels of signaling neuropeptides and chaperones and processing proteases for packaging of self-aggregating peptides into dense core vesicles. Subgroup specific candidate biomarkers were identified for TD PD and PD patients with leukocytes detected in CSF.

Conclusions: PD, MSA and PSP exhibit overlapping as well as distinct protein biomarkers that suggest specific molecular etiologies. This indicates common sensitivity of certain populations of selectively vulnerable neurons in the brain, but also distinct therapeutic targets for PD subgroups. This study validates a decrease in CSF levels of self-aggregating neuropeptides in parkinsonian disorders and supports the role of native amyloidogenic proteins in etiologies of neurodegenerative diseases.
Aims: A key pathological feature of Parkinson’s Disease (PD) is the progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta. The transcription factor Engrailed1 (EN1) is crucial for the development and survival of these neurons and has a neuroprotective effect in a PD MPTP mouse model. En1 knockout mice are an interesting model to study the pathology of PD, as animals heterozygous for En1 show slow degeneration of dopaminergic neurons starting 6 weeks after birth. However, a human model is still missing. To elicit the role of EN1 in the development and survival of dopaminergic neurons in a human model, homozygous and heterozygous EN1 knockout induced pluripotent stem cell (iPSC) lines were generated.

Methods: The CRISPR/Cas9 technology was used to induce insertions/deletions in Exon 1 of EN1 resulting in premature termination of translation.

Results: The EN1+/− and EN1−/− iPSCs have normal colony morphology and possess pluripotent capacity shown by trilineage differentiation. All selected EN1+/− and EN1−/− clones exhibit a normal karyotype showing no structural variation at a detectable resolution of 3 MB. Following quality control, the EN1 knockout cells were successfully differentiated into neuronal progenitor cells with midbrain identity and dopaminergic neurons.

Conclusions: Heterozygous and homozygous EN1 knockout iPSC lines have been successfully generated. The iPSCs have a normal karyotype, are pluripotent and can be differentiated into dopaminergic neurons. The impact of the EN1 knockout is now being analyzed at a functional level at different stages during differentiation in this new human model system.
AN ALL IPSC-DERIVED CORTIO-STRATIO-NIGRAL MINI-CIRCUIT USING CUSTOM MICROFLUIDICS

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Aims: Medium spiny neurons (MSNs) are the most abundant neurons in the striatum and receive inputs from both glutamatergic and dopaminergic afferents. Modelling MSNs in vitro using induced pluripotent stem cells (iPSCs) has been challenging mainly due the lack of physiological maturity which could be in part due to the lack of the complex the connecting partners. By building a cortico-striato-nigral minicircuit, we can better recapitulate the complex connectivity received by striatal neurons and increase the maturation of in vitro iPSC-derived MSNs rendering a better model for the research community.

Methods: Dopaminergic neurons (DaNs), cortical neurons (CNs) and MSNs were differentiated from iPSCs and then sequentially seeded onto custom 3-chamber microfluidic devices. We used whole cell patch-clamp electrophysiology with post-hoc labelling to specifically record the electrophysiological properties of these neurons and examined spine morphology of the MSNs in parallel in the minicircuit.

Results: We established an optimised protocol to generate iPSC-derived MSNs which expressed their canonical protein markers and exhibited MSN-like electrophysiological properties. Coculture of iPSC-derived MSNs with iPSC-derived CNs modelling the corticostratal pathway significantly improved striatal electrophysiological properties and spine morphology. Preliminary data from further addition of DANs to recapitulate the dual glutamatergic and dopaminergic inputs received by MSNs suggested more electrophysiological changes in iPSC-derived striatal neurons.

Conclusions: Our results highlight the utility of modelling striatal neurons in a highly physiological manner preserving their endogenous connectivity, and provides a framework for future minicircuit disease modelling using microfluidics. This advanced model could offer new insights on the disease pathophysiology of complex human neurological conditions.
MODELLING ALPHA-SYNUCLEIN AGGREGATION AND NEURODEGENERATION WITH FIBRIL SEEDS IN PRIMARY CULTURES OF DOPAMINERGIC NEURONS

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Aims: Our present aim was to get better insights into mechanisms underlying α-Synuclein (aS) aggregation and aS-mediated neurodegeneration in Parkinson disease (PD).

Methods: For that, we used midbrain cultures of mouse dopamine (DA) neurons exposed chronically to fibrils 91 generated from recombinant wild-type aS.

Results: We established that fibrils 91 have an exquisite propensity to seed aggregation of endogenous aS in DA neurons (somas + neurites). Inhibitors of DA synthesis had no impact on aS aggregation, suggesting that neither the neurotransmitter nor its metabolites contributed to this process. Up to 2 weeks post-exposure to fibrils, somal aggregation in DA neurons was strictly dependent on fibrils 91 concentrations (0.01-0.75 μM) and time elapsed (1-2 weeks) after fibril exposure. DA cell loss was not observable under these conditions. Besides, neither toxin- nor genetically-induced mitochondrial deficits augmented the vulnerability of DA neurons treated with fibril seeds. Noticeably, DA cell loss was detectable 3 weeks following fibril exposure, i.e., at a stage where somal aggregation had reached a plateau phase. This loss was preceded at 2 weeks post-seeding by early deficits in DA uptake, a sensitive marker of DA cell function. The loss of DA neurons (but not aS aggregation) was prevented by treatment with the trophic factor GDNF, suggesting that the aggregation process in DA neurons may reproduce a form of programmed cell death that mimics a state of trophic factor deprivation.

Conclusions: Overall, we suggest that our model system might be useful to explore PD-related pathomechanisms and therapeutic approaches for this disorder.
HIGHLY ENRICHED HIPSC- DERIVED MIDBRAIN DOPAMINERGIC NEURONS ROBUSTLY MODELS PARKINSON’S DISEASE

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Aims: Develop a protocol to produce hiPSC-derived midbrain dopaminergic neurons which is rapid, efficient, cost effective, and generates highly enriched cultures to model synucleinopathies in vitro.

Methods: We developed a protocol to differentiate hiPSCs into enriched populations of mDA neurons using only small molecules in a timely fashion, to pattern hiPSCs to mDA neurons. We investigated the cellular pathology using single cell imaging and super-resolution microscopy.

Results: Single-cell RNA-sequencing reconstructed the temporal transcriptomic sequence of cellular fates, confirming the development trajectory of neural precursor cells (NPC) into mDA neurons, and identified key driver genes, STMN2, DCX, and SYT1, for midbrain neuronal differentiation. Within 4 weeks of terminal differentiation mDA neurons synthesise and secrete dopamine, exhibit electrical activity, form functional synapses and networks in vitro. Mutations in SNCA cause autosomal dominant Parkinson’s disease, through poorly defined mechanisms. Utilising patient derived hiPSCs with SNCA point mutations and rearrangements, we investigated the temporal sequence of pathophysiological events in mDA synucleinopathies. Adopting super resolution approaches, we discovered the earliest accumulation of abnormal protein assemblies, in the form of beta-sheet rich oligomeric aggregates of α-synuclein. The toxic aggregates subsequently lead to early calcium dysregulation and calcium buffering. This is then followed by abnormalities in mitochondrial calcium, impaired respiration, oxidative stress, and at later stages, upregulation of mitophagy and autophagy, and ultimately cell death.

Conclusions: Our efficient differentiation paradigm to generate enriched mDA neurons provides a robust model for PD. Protein misfolding is the earliest critical event in human neurons, and the accumulation of oligomeric features induces stress and death.
Aims: Accumulation of misfolded alpha-synuclein, forming Lewy-bodies, causes mitochondrial stress and impairs autophagy-lysosomal pathway in Parkinson's disease. Mutations on GBA is a strong risk factor for Parkinson's disease. Here, we developed novel models for studying GBA-linked lysosomal dysfunctions and α-Syn toxicity in Parkinson's disease.

Methods: Primary cultures of mesencephalic neurons were treated with conduritol B epoxide (CBE, 20 µM), an inhibitor of GBA, and were injured with an αSyn solution (250 nM) containing protofibrils. Survival of dopaminergic neurons, neurite network and accumulation of lysosomes were evaluated by immunocytochemistry. Aged mice age were bilaterally injected in the substantia nigra with αSyn protofibrils and treated with CBE (50 mg/kg/2days). After anesthesia and PBS/PFA perfusion, brains were dissected. Survival of dopaminergic neurons in the substantia nigra, alpha-synuclein aggregation and microglia activation were investigated by immunohistochemistry.

Results: In vitro toxicity of αSyn on dopaminergic neurons was exacerbated by the inhibition of GBA. Ambroxol hydrochloride, a GBA chaperone, reduced lysosomal burden in primary dopaminergic neurons. In vivo, a progressive loss of dopaminergic projections and neurons in the substantia nigra was observed after intra-nigral injections of alpha-synuclein and CBE treatment. Neuronal loss was associated with alpha-synuclein aggregation and neuroinflammation. Mice presented also with gait impairments. In absence of CBE, the toxicity of αSyn on dopaminergic neurons was reduced.

Conclusions: Altogether, we show here that inhibiting GBA activity exacerbates the toxicity of alpha-synuclein protofibrils on dopaminergic neurons, in in vitro and in vivo models of GBA-linked Parkinson's disease.
LINEAGE TRACING OF SINGLE CELL RNA-SEQUENCING DATA FROM HUMAN MIDBRAIN IDENTIFIES NOVEL GENES AFFECTED BY IDIOPATHIC PARKINSON'S DISEASE

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Aims: Hundreds of genes are associated with idiopathic Parkinson's Disease (PD) from Genome-Wide Association Studies (GWAS). However, it is unclear if cell type specific expression of PD GWAS genes are associated with progression of disease, or if other genes may be driving disease progression. Using a single cell lineage tracing algorithm we found genes associated with PD - and potentially PD progression - that have not been previously implicated by GWAS.

Methods: We used Partition-based Graph Abstraction (Wolf et al. 2019) together with variational auto-encoders (Gayoso et al. 2021) to learn low-dimensional data representation based on single cell RNA-sequencing data from human midbrain (Kamath et al. 2021). We analyzed ~17 000 dopaminergic cells collected from 13 patients (7 healthy controls vs 6 PD patients). We identified genes that were upregulated and downregulated along the pseudo-time trajectories.

Results: We found a set of 160 genes (40 genes per 4 trajectories, FDR-adjusted p<0.05) that are substantially affected by Parkinson's disease, i.e. their activity is correlated with disease associated trajectory. To validate our results we superimposed the inferred pseudo-time estimates with patient metadata and found a positive correlation between the disease pseudotime and patient metadata (r=0.25, Pearson coefficient). Using enrichR and search in BioPlanet database 2019 we found that identified gene sets are related to the axon guidance and developmental biology (p=6.89*e-07, p=1.25e-06, FDR-adjusted p<0.05).

Conclusions: Our results provide novel insights into the pathophysiological processes associated with idiopathic Parkinson's disease and would help to find better drug targets to reverse the pathological changes in gene expression.
Aims: Accumulation of abnormal protein aggregates is the characteristic of neurodegenerative diseases. Pathological aggregates spread progressively from specific brain regions to larger areas as the diseases progress in the brain. Since Aggregate transmission is the underlying a major mechanism for pathological propagation, understanding the mechanism of inter, trans-cellular transmission of aggregates would be a promising strategy to slow down the progression of disease.

Methods: In this study, we established a high-contents screening (HCS) system to identify the modifier that regulate disease progression, and performed adaptation processes the C. elegans model to the HCS system.

Results: We confirmed that the validated genetic factors, associated with aging, lysosomal function, and cellular trafficking, regulate the rate of aggregate propagation and generated C. elegans models for RNAi screening in the automated screening system.

Conclusions: These results show that we constructed an HCS system capable of measuring the change in fluorescence intensity quantitatively in the C. elegans model, and it is suggested that the thus can be used to screen and identify a factors that regulate the transfer of protein aggregates.
MULTIOMIC LANDSCAPING OF PARKINSON’S DISEASE POSTMORTEM MIDBRAINS

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Aims: In this study we aimed to profile the molecular landscape of Parkinson’s disease (PD) postmortem midbrains using a set of multiomics approaches. Furthermore, we aimed to obtain a more integrative view of disease-mediated alterations in late-stage PD by exploring the deregulated networks captured across different omics layers.

Methods: Tissue from 13 PD patients and 10 controls was obtained from the Parkinson’s UK Brain Bank and subjected to multiomic analyses: small and total RNA sequencing were performed on the Illumina’s HiSeq4000, while proteomics was performed in the TripleTOF5600+ mass spectrometer following data-independent acquisition mass spectrometry (DIA-MS). Differential expression analyses were performed with customized frameworks based on DESeq2 and with Perseus(v.1.5.6.0). Custom pipelines in R were used for integrative studies.

Results: Our analyses revealed multiple deregulated molecular targets linked to known pathomechanisms in PD, as well as novel processes. Differential expression analyses identified miR-539-3p, miR-376a-5p, miR-218-5p, and miR-369-3p, valid miRNA-mRNA interacting pairs (miR-218-5p/RAB6C; miR-369-3p/GTF2H3), and multiple regulated proteins (CHI3L1; SELENBP1; PRDX1; HSPA1B; TH). Vertical integration of multiomic analyses allowed to validate disease-mediated alterations across different molecular layers. Functional annotation of differentially expressed molecules showed an enrichment of pathways related to neuroinflammation, mitochondrial dysfunction and defects in synaptic function.

Conclusions: This comprehensive and integrative assessment of PD-affected and control human midbrains revealed multiple molecular targets and networks that are relevant to the disease mechanism of advanced PD. Our analysis suggests neuroinflammation, immune response activation, mitochondrial and synaptic dysfunction as putative therapeutic targets for advanced PD.
Aims: This study aimed to identify and molecularly characterize the target gene underlying a chromosome 7 locus (sentinel SNP rs199347) linked to Parkinson’s Disease (PD) by genome-wide association studies (GWAS).

Methods: We used colocalization analyses of the expression quantitative trait locus (eQTL) and PD risk signals, corroborated by capture RNA-seq-based allele specific expression (ASE) analyses of human brain samples, to nominate the most likely target gene for the rs199347 locus. We then investigated protein-protein interactions between the nominated target gene and alpha-synuclein (aSyn) using subcellular co-localization and co-immunoprecipitation. By genome-editing iPSC-derived neurons (iPSC-N), we characterized the effects of deletion of the target gene on aSyn and the synapse through confocal microscopy, and on the transcriptome through RNA-seq. Finally, we investigated protein levels of the target gene in plasma and CSF from 781 PD and 59 neurologically normal control individuals.

Results: Colocalization and ASE studies nominated glycoprotein non-metastatic melanoma protein B (GPNMB) as the target gene underlying risk for PD, with higher GPNMB expression associated with increased PD risk. In immortalized cell lines, GPNMB co-immunoprecipitated and co-localized with aSyn. In iPSC-N, deletion of one or two copies of GPNMB resulted in loss of aSyn from the synapse, and transcriptomic profiling of GPNMB-deleted lines revealed profound effects on the synapse. Compared to controls, PD patients had higher GPNMB levels in the plasma; within PD, higher plasma GPNMB associated with greater motor severity.

Conclusions: Computational, cell biological, and human tissue-based studies establish GPNMB as a GWAS-derived risk gene for PD, mediating pathogenicity through interactions with aSyn.
Aims: Parkinson’s disease (PD) is characterised by the degeneration of dopamine neurons (DA�s). Calcium is crucial in DA�s, where continuous calcium waves occur. Therefore even small alterations in calcium homeostasis might be deleterious for DA�s. Here we have focused our attention on the role of intracellular calcium in the pathogenesis of PD.

Methods: -Induced pluripotent stem cell-derived DA�s from controls and patients. -Fura-2 AM and genetically encoded calcium indicators entrapped in the ER (CEPIAer) and the mitochondria (R-GECOmt) for calcium imaging -Proximity ligation assay (PLA) to measure the interaction between the calcium channels IP3R and VDAC1 and between the two calcium channels and α-synuclein

Results: SNCA-Trp and GBA-N370S DA�s display decreased intracellular calcium release in a Fura-2 AM-based assay as well as decreased ER calcium release upon ionomycin stimulation. SNCA-Trp and GBA-N370S DA�s also display decreased mitochondrial calcium uptake upon ionomycin stimulation. The observation of an alteration in calcium dynamics between the ER and the mitochondria of PD patient derived DA�s, might suggest an alteration in the function of ER-mitochondria contact sites. Therefore, we have recently been studying ER-mitochondria contact sites using PLA. Preliminary results suggest that IP3R-VDAC1 and VDAC1-α-synuclein interactions might be decreased in SNCA-Trp DA�s.

Conclusions: Studying calcium dynamic deficits in iPSC-derived DA�n models of PD could shed light on the pathogenic mechanisms associated with neuronal vulnerability and death in PD. In particular, investigating the involvement of ER-mitochondria contact sites in the altered calcium dynamics described, will bring us closer their cause, with the ultimate goal to identify new therapeutic compounds for PD.
POST-MORTEM QUANTIFICATION OF CORTICAL GLIA CELLS IN PATIENTS WITH PARKINSON’S DISEASE

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Aims: Parkinson’s disease (PD) is a debilitating neurodegenerative disorder, characterized by motor and non-motor symptoms. Increasing evidence suggest that the neocortex is involved in both disease characteristics, and neocortical neuroinflammation have been reported previously, among other changes. However, the impact of PD on the number of neocortical cells is sparsely studied. In the present study, the aim was to quantify the total number of neocortical neurons- and glial cells (astrocytes, oligodendrocytes and microglia) in PD patients compared to control subjects.

Methods: We used stereological methods to estimate the total number of neocortical neurons, oligodendrocytes, astrocytes and microglia in frontal-, temporal-, parietal and occipital cortices of brains from 10 PD patients and 12 control subjects.

Results: In the entire neocortex, there were no significant differences in any cell counts between patients with PD and control subjects. The number of astrocytes and microglia were also without significant difference between groups in all studied regions, a finding that argues against severe neocortical neuroinflammation in PD patients. Finally, a significant decrease of 35% in the number of oligodendrocytes was observed in the frontal cortex of patients with PD.

Conclusions: In conclusion, future studies are warranted to clarify the functional relevance of oligodendrocytic loss in PD. As this study exclusively examined neocortical areas, it would be of particular interest to study white matter oligodendrocytes for further elaboration of our understanding of PD pathology.
POSTERS

EXAMINATION OF NEURONAL CELL MODELS AS TOOLS FOR THE EVALUATION OF NEW THERAPEUTIC STRATEGIES FOR PARKINSON’S DISEASE

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Aims: Examine physiologically relevant cellular models for preclinical research and drug development for Parkinson’s Disease (PD). Selecting appropriate cellular models is highly valuable for mechanism of action studies of novel therapeutic agents as part of the preclinical efficacy and safety data package before entering clinical development.

Methods: To model the main cellular pathophysiology in PD, three different cell types were differentiated into dopaminergic (DA) neurons. Lund Human Mesencephalic (LUHMES) cells were differentiated into DA neurons using a cocktail of neurotrophic factors, pleiotropic cytokines, and antioxidants. Patient-derived induced pluripotent stem cells (iPSCs) were first differentiated into neuronal progenitors by activating molecular pathways that guide the DA neuron formation in vivo, using a cocktail of transcription factors, activators of sonic hedgehog (SHH) and canonical WNT signalling. SH-SY5Y cells were differentiated into neuronal cultures by subjecting them to conditions of serum deficiency, retinoic acid and neurotrophic factors.

Results: Neuron-like morphology was observed in all differentiated cell cultures by microscopic examination. Expression of neuronal (TUBIII) and dopaminergic markers (TH, NURR1, FOXA2) was reported in the differentiated cell models by RT-qPCR. Differentiated LUHMES and iPSC-derived cell lines were characterized by flow-cytometry showing a positive expression of TUBIII in 57.2% and 16.9% of the cell population, respectively.

Conclusions: Altogether, these results validate the methods used for the differentiation of LUHMES, iPSCs and SH-SY5Y cells into neuronal cultures, indicate differences in their efficiency to obtain a pure neuronal model, and confirm their potential use as in vitro models for the evaluation of new therapeutics for PD.
PRECLINICAL DEVELOPMENT OF SMALL-MOLECULE INHIBITORS OF NON-AMYLOIDOGENIC AGGREGATION OF ALPHA-SYNucleIN IN PARKINSON’S DISEASE

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Aims: Non-amyloidogenic, alpha-synuclein oligomers are key features and a target for the design of disease modifying therapies in Parkinson’s Disease (PD). This work aimed to study the toxicity of alpha-synuclein (α-syn) over time and investigate the ability of two drug repurposing compounds to prevent this effect.

Methods: To study the efficacy and safety of the anti-oligomerization compounds and evaluate the toxicity of α-syn incubated for different days, we used cellular assays such as MTT and caspase-3 kits, immunocytochemistry with phalloidin labelling, and morphological analysis, and used two cell lines, the human neuroblastoma SH-SY5Y, and the rat adrenal phaeochromocytoma PC12.

Results: Our in vitro results demonstrated that for both compounds, concentrations below 5 μM and 15 μM are safe to be used in PC12 and SH-SY5Y cells, respectively, as evaluated by the MTT viability assay. We also demonstrated that the incubation of SH-SY5Y cells with α-syn lead to the formation of species that dysregulated cell cytoskeleton and activated apoptotic cell death mechanisms, which were counteracted by one of the tested compounds, suggesting that this compound plays a protective role.

Conclusions: A drug repurposing candidate was identified with a protective effect on the cell toxicity induced by non-amyloidogenic α-syn oligomers.
POSTERS

A BRAIN-PENETRANT STEAROYL-COA DESATURASE INHIBITOR REVERSES A-SYNUCLEIN TOXICITY IN CELLULAR SYNUCLEINOPATHY MODELS

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Aims: To evaluate efficacy of YTX-7739, a brain-penetrant stearoyl-CoA desaturase (SCD) inhibitor, in various cellular models of synucleinopathies.

Methods: Efficacy of YTX-7739 was profiled in the following cellular α-synuclein (αSyn) toxicity paradigms: 1. Survival assay measured by longitudinal tracking of αSyn over-expressing human neurons; 2. Inclusion formation or αSyn phosphorylation in M17D cells and human neurons expressing familial E46K αSyn (or amplified 3K) mutation; 3. Fatty acid desaturation index (FADI) and phosphorylated αSyn levels in PD patient-derived 3D cortical neurospheres.

Results: YTX-7739 decreased αSyn-mediated neuronal death measured by longitudinal survival analysis. In both M17D cells and human neurons, YTX-7739 reversed the abnormal membrane interaction of 3K αSyn and decreased the phosphorylation of E46K αSyn. Moreover, YTX-7739 reversed pathological phenotypes in A53T and αSyn triplication patient-derived cortical neurospheres, including dysregulated fatty acid profiles and pS129 αSyn accumulation.

Conclusions: Together, these data provide validation of SCD as a therapeutic target, and YTX-7739 as a clinical candidate, for treating human α-synucleinopathies.
STABILIZATION OF MONOMERIC α-SYNUCLEIN BY ALL-D-ENANTIOMERIC PEPTIDE LIGANDS AS THERAPEUTIC STRATEGY FOR PARKINSON’S DISEASE AND OTHER SYNUCLEINOPATHIES

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Aims: In Parkinson’s disease oligomeric structures of α-synuclein are thought to play a key role in cell-to-cell transmission and induction of toxic effects. Here, we report the development of all-D-enantiomeric peptide ligands that bind monomeric α-syn with high affinity, thereby stabilizing the physiological intrinsically disordered structure and preventing initiation of aggregation as well as eliminating already existing aggregates.

Methods: Mirror image phage display, next-generation sequencing (NGS), Thioflavin-T assay (ThT), 2D-NMR, surface plasmon resonance (SPR), microscale thermophoresis (MST), density gradient centrifugation (DGC), quantitative HPLC, cell viability assay (MTT), intracellular aggregation assay.

Results: Based on mirror image phage display on the D-enantiomeric full-length α-syn target, we identified two lead compounds SVD-1 and SVD-1a by NGS, ThT screens and rational design. The compounds were analyzed with regard to their anti-aggregation potential, where both compounds showed aggregation delaying as well as seed capacity reducing effects in de novo and seeded environments, respectively. High affinity towards the monomeric α-syn, in the low nano- to picomolar Kᵤ range was identified by SPR as well as MST assays, whereas no impact on the chemical environment of the random coil structure was observed in 2D-NMR experiments. Finally, SVD-1 as well as SVD-1a reduced toxic effects of soluble PFF seeds in cell culture and SVD-1a was able to specifically eliminate α-syn oligomers as identified by density gradient centrifugation and quantitative HPLC.

Conclusions: The present work provides promising results on the development of D-enantiomeric peptide lead compounds with an anti-prionic mode of action for future treatment of Parkinson’s disease and other synucleinopathies.
Aims: To evaluate in vivo pharmacology and efficacy of YTX-7739, a clinical-stage stearoyl-CoA desaturase (SCD) inhibitor in development for Parkinson's disease.

Methods: YTX-7739 was administered to mice, rats, and cynomolgus macaques and both pharmacokinetics and pharmacodynamics assessed. Fatty acid profiles were analyzed after repeated dosing. YTX-7739 was administered to a transgenic mouse model expressing an amplified fE46K “3K” Parkinson’s disease mutation in α-synuclein. Both pharmacology and amelioration α-synuclein-dependent pathologies were assessed.

Results: YTX-7739 exhibited favorable pharmacokinetic properties and reduced levels of monounsaturated fatty acid SCD products across a range of tissues and biofluids. Studies characterized response specificity, kinetics, and translation of pharmacology from rodents to cynomolgus monkeys. There was a significant correlation between plasma and brain pharmacology, indicating that changes in plasma reflect comparable changes in brain. These data established dosing paradigms to assess a transgenic mouse model expressing an amplified fE46K “3K” Parkinson’s disease mutation. Reduction of the fatty acid desaturation index restored the α-synuclein tetramer:monomer ratio, reduction in pS129 α-synuclein, reduced proteinase-resistant and lipid droplet-rich aggregates, increased survival of dopaminergic and cortical neurons, and reversal of motor deficits. Genetic validation by deleting one SCD allele afforded comparable results to YTX-7739.

Conclusions: The robust pharmacology and efficacy associated with SCD inhibition by YTX-7739 support and inform on-going clinical evaluation of YTX-7739 as a disease-modifying drug for synucleinopathies. Importantly, the strong correlation between plasma and brain pharmacology establishes plasma as a suitable matrix to assess target engagement in human clinical studies.
INTEGRATED STRESS RESPONSE IN ALPHA-SYNUCLEINOPATHY

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Aims: Abnormalities in α-synuclein (αS) is directly linked to the pathogenesis of Parkinson’s disease (PD) and related disorders called a-synucleinopathies. We showed that α-synucleinopathy in a αS transgenic model (TgA53T) and humans causes chronic endoplasmic reticulum stress (ERS)/unfolded protein response (UPR)/Integrated Stress Response (ISR). Treatment of TgA53T with salubrinal, a compound that inhibits dephosphorylation of eIF2α and attenuates ERS induced toxicity, can significantly delay onset of αS pathology and motor deficits. To further establish ERS/UPR/ISR as an important pathogenic factor in a-synucleinopathy, we examined if reducing phosphorylation of eIFα by the Protein kinase R-like ER Kinase (PERK), exacerbates a-synucleinopathy.

Methods: Immunoblot analysis, HIC and IF tissue staining were used for analysis of mice tissue. Sample sizes are based on data from prior studies and calculated to provide 80% power to detect >15% differences in mean.

Results: We show that conditional deletion of PERK in neurons of TgA53T mice leads to significantly earlier onset of a-synucleinopathy, showing the pathologic importance of PERK-eIF2α pathway. While salubrinal inhibits both the protein phosphatase 1 regulatory subunit 15A (PPP1R15A, Gadd34) and PPP1R15B (CReP), studies show that inhibition of Gadd34 could be neuroprotective. Significantly, neither the pharmacological inhibition of Gadd34 or genetic loss Gadd34 function attenuated a-synucleinopathy in TgA53T model. Significantly, treatment of TgA53T model with an CReP inhibitor delays disease onset and attenuates pathology in TgA53T model.

Conclusions: Our data suggest ISR components, particularly inhibition of CReP, is a therapeutic target for α-synucleinopathy.
Aims: The mRNA for a-synuclein encodes a uniquely folded version of an iron-responsive element (IRE) RNA stem loop. We showed that the drug Posiphen inhibited SNCA mRNA translation by targeting its’ 5’untranslated region in the micromolar range to lower a-syn levels ex vivo and in vivo. Our aims are to generate proof-of-principle that the 5’untranslated region of the SNCA transcript can be a highly selective target to identify inhibitors of alpha-synuclein (a-syn) and to compare our novel 5’UTR SNCA inhibitors to Posiphen.

Methods: We conducted a high throughput screen and characterized selective SNCA mRNA directed translation blockers (incl. Syn-516) (PUBCHEM AID 2627). We used SNCA 5’UTR-luciferase reporter constructs, ELISA and western blotting secondary assays, Direct RNA binding by Tm calorimetry and 11C labeling and PET imaging in mice to evaluate BBB penetrability.

Results: The Syn-516 blocker probe, and 3 additional selective SNCA 5’UTR inhibitors A3, B1 and C1, exhibited potent inhibition of a-syn translation. These SNCA 5’UTR inhibitors significantly decreased a-syn levels by more than 50% in primary iPSC derived dopaminergic (DA) neurons. Syn-516 also reduced a-syn in iPSC derived cholinergic neurons expressing the triple SNCA gene. Each inhibitor directly bound to RNA oligos encoding the SNCA 5’UTR RNA sequences by Tm calorimetry. Inhibitors B1 and C3 were labelled with 11C and demonstrated BBB penetrability using PET imaging.

Conclusions: The Syn-516 blocker probe, and 3 additional selective SNCA 5’UTR inhibitors exhibited comparable and greater efficacy to Posiphen to inhibit alpha Syn translation. Our data support rapid further testing of these inhibitors for their ADMET properties for advancement into human clinical trials.
DEVELOPMENT OF C-TERMINAL A-SYNUCLEIN VACCINE FOR TREATMENT AND PREVENTION OF PARKINSON’S DISEASE AND OTHER SYNUCLEINOPATHIES

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Aims: Parkinson’s Disease (PD) and other synucleinopathies are characterized by pathological accumulation of α-synuclein in both CNS and peripheral neurons resulting in disease progression. Prasinezumab a humanized monoclonal antibody targeting the C-terminal region of α-synuclein, showed signals of efficacy on multiple prespecified secondary and exploratory clinical endpoints. Prothena developed vaccines targeting epitopes identified in clinical and/or preclinical studies that may slow disease progression for the potential treatment and prevention of PD and other synucleinopathies.

Methods: Linear single and tandem α-synuclein peptides were synthesized and conjugated to CRM 197. Mice and guinea pigs were injected with conjugate and QS21 adjuvant. Sera from immunized animals were titered against both recombinant and pre-formed fibrils of α-synuclein. Fresh frozen PD and control brains were used to confirm specificity of binding to pathological α-synuclein inclusions. Antibody potency was assessed in vitro by blocking cellular uptake of aggregated alpha-synuclein in B103 cells.

Results: We identified a lead vaccine candidate with C-terminal α-synuclein peptides positioned in tandem that produced sera with higher serum titers, greater binding to pathological α-synuclein inclusions in PD patient brains, and a stronger ability to inhibit the uptake of pathogenic synuclein aggregates into B103 cells when compared to a vaccine containing a single peptide.

Conclusions: We developed a tandem vaccine that raises high titers of antibodies to the C-terminus region of α-synuclein, with robust binding to pathogenic α-synuclein and inhibition of uptake of soluble α-synuclein aggregates into cells. This novel approach could lead to a vaccine for both treatment and prevention of Parkinson Disease and other synucleinopathies.
DEVELOPMENT OF USP30 INHIBITORS AS DISEASE MODIFYING THERAPEUTIC FOR PARKINSON’S DISEASE

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Aims: No disease modifying therapeutics currently exist for Parkinson’s disease (PD), and despite strong genetic evidence from monogenic mutations in PINK1, parkin, and FBXO7, as well as risk factors for idiopathic PD, there have been no efforts in clinical trials to enhance mitophagy as a therapeutic approach. Several lines of evidence suggest that deficits in mitophagy are a shared feature of genetic and idiopathic forms of PD. USP30 has recently emerged as a key regulator of mitochondrial clearance in opposition to the actions of parkin to drive ubiquitination and clearance of depolarized mitochondria.

Methods: We have developed proprietary compounds with low nanomolar in vitro potency for USP30 inhibition. The compounds are cell-penetrant and enhance mitophagy in the presence of antimycin/oligomycin (A/O) in human neural cells with endogenous expression of USP30, parkin, and substrates. Importantly, the compounds do not damage or depolarize healthy mitochondria as measured by TMRE. Lead compounds are highly selective when tested against a panel of over 40 deubiquitinating enzymes using two orthogonal assays.

Results: We have profiled compounds for various ADME properties and successfully optimized properties including solubility, permeability, microsomal stability, and plasma protein binding. Our lead compound’s pharmacokinetic profile in rats and mice demonstrates brain penetration at levels well above 2x the low nM IC50 setting the stage for invivo POC studies.

Conclusions: Enhanced clearance of damaged mitochondria holds promise for PD and other age-related diseases and Vincere’s proprietary potent USP30 inhibitors are well positioned for further development.
NICOTINE-MEDIATED RECRUITMENT OF GABAERGIC NEURONS TO A DOPAMINERGIC PHENOTYPE ATTENUATES MOTOR DEFICITS IN ALPHA-SYNUCLEIN PARKINSON’S MODEL

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Aims: Previous work revealed an inverse correlation between smoking and Parkinson's disease (PD) that is associated with nicotine-induced neuroprotection of dopaminergic (DA) neurons against nigrostriatal damage in PD primates and rodent models. Nicotine, a neuroactive component of tobacco, can directly alter the activity of midbrain DA neurons and induce non-DA neurons in the substantia nigra (SN) to acquire a DA phenotype. We investigated the recruitment mechanism of nigrostriatal GABAergic neurons to express DA phenotypes, such as transcription factor Nurr1 and DA-synthesizing enzyme tyrosine hydroxylase (TH), and the concomitant effects on motor function.

Methods: Wild-type and α-syn-overexpressing (PD) mice treated with chronic nicotine were assessed by behavioral pattern monitor (BPM) and immunohistochemistry/in-situ hybridization to measure behavior and the translational/transcriptional regulation of neurotransmitter phenotype following selective Nurr1 overexpression or DREADD-mediated chemogenetic activation.

Results: Nicotine treatment led to a transcriptional TH and translational Nurr1 upregulation within a pool of SN GABAergic neurons in wild-type animals. In PD mice, nicotine increased Nurr1 expression, reduced the number of alpha-syn-expressing neurons, and simultaneously rescued motor deficits. Hyperactivation of GABA neurons alone was sufficient to elicit de novo translational upregulation of Nurr1 in non-DA neurons. Retrograde labeling revealed that a fraction of these GABAergic neurons projects to the dorsal striatum.

Conclusions: Nicotine exposure initiates neuroprotective mechanisms counteracting the neurodegenerative effects of α-syn accumulation in DA neurons and contributing to Nurr1-mediated therapeutic effects. Revealing the mechanism of nicotine-induced DA plasticity protecting SN neurons against nigrostriatal damage could contribute to developing new strategies for neurotransmitter replacement in PD.
Aims: Ketamine has been shown to be anti-dyskinetic in preclinical models of Parkinson’s Disease (PD). This study does investigate the role of ketamine in reducing neural signatures of Levodopa-Induced Dyskinesia (LID) in a rat model of PD.

Methods: We induced LID in 6-hydroxydopamine-lesioned (6-OHDA) rats by treating animals regularly with L-DOPA (12 mg/kg for ≥10 days). During each experimental session, control and LID-expressing rats were injected with saline or L-DOPA (12 mg/kg, i.p.; subjects: n=1 control, n=1 6-OHDA, n=8 total sessions). Injections were followed 1-hr later by a ketamine injection (20 mg/kg, i.p.). Single-unit and local-field responses were measured from each hemisphere through a dual-bundle 16-tetrode hyperdrive (AP: 1.5, ML: +/-2.2mm).

Results: Preliminary data indicate that LID-associated 80-Hz oscillations and dyskinetic movements were suppressed within 5 min following ketamine injection, with the 80-Hz oscillations being replaced by robust low-gamma (~50 Hz). Burst firing in M1 neurons (n=288) were reduced in the naïve animal but no significant changes were observed in M1 neurons of the lesioned (n=93) or un-lesioned (n=201) hemispheres in LID.

Conclusions: Our preliminary data indicate that ketamine reduces 80-Hz oscillations and dyskinetic behavior in LID. Interestingly, ketamine reduced bursting in the naïve but not in the LID animal, suggesting that long-term dopamine depletion or exposure to L-DOPA alters ketamine’s capacity to alter the spike timing statistics of M1 neurons. These preclinical studies further complement current ongoing clinical testing of sub-anesthetic ketamine for the treatment of LID by our group, and provide further evidence in support of repurposing ketamine to treat individuals with PD.
Aims: Previously, we demonstrated safety evidence when autologous peripheral nerve tissue (PNT) is deposited in basal ganglia locations in conjunction with standard deep brain stimulation surgery (DBS). In the current pilot study, we examined cognitive and motor outcomes in a Parkinson’s disease (PD) group following bilateral globus pallidus interna (GPI) DBS plus nucleus basalis of Meynert (NBM) PNT. We hypothesized that regenerative PNT delivery to the NBM would support cholinergic cell survival resulting in stable cognitive outcomes.

Methods: Outcome data were analyzed for 7 participants with bilateral GPI DBS plus unilateral NBM PNT. Post-DBS neurocognitive evaluations were on average 12.40 months post-surgery (SD = 3.05). Pre- to post-DBS cognitive scores were compared via paired sample t-tests. On- and off-state Unified Parkinson’s Disease Rating Scale (UPDRS) scores were obtained 12 months post-surgery. Two participants died of unrelated causes >12 months post-grafting followed by pathology examination.

Results: No significant changes were noted in verbal memory, working memory, processing speed, or executive functions; phonemic fluency neared significance (FAS t = 2.295; p = 0.061). Twelve-month UPDRS Part III OFF-state scores indicated no change from enrollment (mean difference = 1.9; 95% CI: -4.6 to +8.3); ON-state scores remained stable (M = 22.0; SD = 2.7). Pathology demonstrated cholinergic fibers extending towards the PNT.

Conclusions: DBS plus NBM participants showed stable cognition. There was no change in motor function, while pathology supported accurate deposition of NBM grafts. Overall, the procedure appears safe with potential for graft-induced preservation of cholinergic neurons coupled with potential cognitive benefit.
Aims: Brain stimulation techniques with transcranial shockwaves are studied as an approach to modulate the human brain in a focal and targeted manner, by demonstrating the efficacy in mild cognitive decline (MCD) and early Alzheimer’s disease (EAD).

Methods: Firstly a visit with a neurologist was set to evaluate the patient and establish a clinical pre-diagnosis of MCD or EAD. Then, cranial MRI and neuropsychological assessment was performed. Neuropsychological battery was made by the Barcelona test before visiting the neurologist to analyze the exams and establish a probable diagnosis. The patient then received 6000 pulses/session: 800 pulses in both frontal areas, 400 pulses on each parietal area and 600 pulses on the precuneus area before repeating the same sequence. (Short pulses of 3 microseconds, 0.2-0.3 mJ/mm²). The treatment duration was 30 minutes 3 times per week, during 2 weeks. 6 months follow-up was performed.

Results: Out of 4 patients, 3 were diagnosed of EAD and 1 of MCD, and only 3 completed the follow-up. All the patients experienced a sustained improvement in attention and fluency of language for at least three months. Shockwaves stimulation is a non-invasive and well-tolerated technique that could replace pharmacological treatment of patients with MCD and EAD. Shockwaves based brain stimulation techniques could stand as an alternative therapy to conventional approaches for Alzheimer’s disease.

Conclusions: Patients had a good tolerability and no side effects. The technique is safe, feasible and efficient and further studies should be performed with an increased sample size.
INSULIN AND SB-216763, A GSK3Β INHIBITOR, PROTECT HUMAN SH-SY5Y AND RAT PRIMARY MIDBRAIN CELLS AGAINST METHAMPHETAMINE TOXICITY

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Aims: Methamphetamine (MA) induces cell death through several mechanisms. Insulin has important roles in the regulation of cell proliferation and apoptosis by GSK3β inactivation. Here, we evaluated the effect of insulin and SB-216763, a selective GSK3β inhibitor, on MA-induced cell death in human neuroblastoma SH-SY5Y and rat primary midbrain cells.

Methods: Human SH-SY5Y and rat primary midbrain cells (extracted from E14.5 rat embryo) were treated with different concentrations of insulin (0.005-0.15U) or SB-216763 (0.5-9µM) with or without MA (5mM). The cell viability was evaluated after 24, 48 and 72 h. The expression of TNFα, Bax, Bim and Bcl2 was examined in primary midbrain cells after 72 h of treatment with 5mM MA, insulin (0.1U) and SB-216763 (3µM).

Results: MA significantly decreased the viability of human SH-SY5Y and rat primary midbrain cells, however, insulin and SB-216763 could increase it. In addition, increase in the expression of TNFα, Bax and Bim following MA, was attenuated by insulin and SB-216763 in primary midbrain cells.

Conclusions: These findings revealed that MA decreases the cell viability of human SH-SY5Y and rat primary midbrain cells, at least in part, by up-regulation of inflammatory and apoptotic factors, and treatment with insulin and SB-216763 could attenuate MA toxicity.
Aims: To date, no neuroprotective/disease-modifying strategy has already been approved as a PD therapy, because of the 'one-disease-one-target' view that has been followed. New drug-based therapeutic routes, namely Safinamide, have been introduced as a promising multimodal drug combining dopaminergic and non-dopaminergic (neuroprotective) actions, representing a new potential alternative therapy to prevent or delay PD progression. Thus, the objective of the present work was to address safinamide impact on PD, relying on the possibility to potentiate TH-positive cells number and tackle some cellular/molecular issues responsible for the failure of dopaminergic neuronal survival.

Methods: Safinamide (10mg/kg) was given by oral gavage to a 6-OHDA rat model. Dopaminergic neuronal survival, neuroinflammation, and redox system homeostasis were assessed by histological and molecular analysis.

Results: We observed that safinamide treatment was able to potentiate the densities of TH-positive cells, revealing a protective effect when compared to the untreated group. To understand possible pathways associated with this improvement, we found that safinamide appears to be a modulator of the antioxidant system and autophagy since an increase in the expression levels of DJ-1, SOD-1, and LC3B was observed when compared to the non-treated group. Additionally, safinamide presents a potential modulatory activity on neuroinflammation and astrogliosis, as a decrease in microglia (CD11b+) and astrocytic (GFAP+) cells number was observed when compared to 6-OHDA group.

Conclusions: Collectively, these data demonstrate the promising therapeutic potential of safinamide as an eventual neuroprotection strategy for PD, which may thus open new therapeutic opportunities for the treatment of this pathology, particularly for its earlier stages.
HIGH-INTENSITY INTERVAL TRAINING IMPROVES SYSTEMIC INFLAMMATION AND ANTIOXIDANTS LEVEL IN IDIOPATHIC PARKINSON'S DISEASE

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Aims: High-intensity interval training is one of the best types of endurance training which allows in a short term to improve the body fit. Moreover, previous years show the importance of interval training as a form of rehabilitation in Parkinson's disease, but studies on the influence of interval training on the inflammation and antioxidants in Parkinson's disease are limited. This research aimed to investigate the influence of 12 weeks of high-intensity interval training on the level of inflammation markers and antioxidants in patients with Parkinson's disease.

Methods: Fifteen patients with diagnosed Parkinson's disease were enrolled in this study as the training group and thirteen people with Parkinson's disease were enrolled as the control group. The level of cytokines (TNF-α, IL-10, IL-6) were determined in the serum by using the ELISA method. Antioxidants activity (glutathione, catalase, SOD) in the serum was determined by using colorimetric kits. Hematological parameters were determined in the whole blood on the hematological analyzer.

Results: show that after 12 weeks of high-intensity interval training, the level of TNF-α, neutrophils/lymphocytes ratio, systemic immune-inflammation index, and neutrophils decreases, with the increases in the level of IL-10 and SOD. Moreover, results show that the level of IL-6 and leukocytes shows a decreasing tendency after interval training.

Conclusions: The results suggested that 12 weeks of high-intensity interval training improves systemic inflammation by a decrease in the TNF-α level and systemic inflammation markers with the increase in the SOD activity. This research was supported by grant no. 2018/31/N/NZ7/02431 from the National Science Center of Poland
NEW FORMULATION OF NADPH OXIDASE INHIBITOR TO PREVENT NEURODEGENERATION IN PARKINSON’S DISEASE.

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Aims: Oxidative stress is one cause of cellular instability and cell death in neurological diseases. The NADPH oxidases (Nox) are an important source of oxidative stress linked to the onset of pathological processes in Parkinson’s Disease (PD). Apocynin (Apo) a non-specific Nox inhibitor, has neuroprotective properties in PD context. Nonetheless, Apo has limited solubility in aqueous solutions, influencing its bioavailability and efficacy. We aimed to reformulate Apo to make it soluble in aqueous solutions and increase its bioavailability and neuroprotective effect in PD context.

Methods: The cytotoxicity and neuroprotection evaluation of the reformulation were performed in N27 dopaminergic cell and in primary cultures of microglia and astrocytes. To test, in vitro, the dopaminergic neuroprotective effect of the reformulation, we used N27 cell exposed to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or 6-hydroxidopamine (6OHDA). The effectiveness in vivo was evaluated in the 6OHDA-based animal PD model.

Results: The results showed a significant increase in the solubility of the reformulation in aqueous solution when compared to non-reformulated Apo. The reformulation did not induce cytotoxic effect on dopaminergic neurons, astrocytes and microglia primary cultures, showing higher EC50 then Apo. The reformulation of Apo was able to prevent 6-OHDA neurotoxicity in vitro, while this was not observed for MPP+. As for the in vivo evaluations, the reformulated Apo did not cause cytotoxicity to SNpc dopaminergic neurons and prevented dopaminergic neurodegeneration induced by 6OHDA.

Conclusions: Apo reformulation has circumventing its solubility problem improving its bioavailability and efficacy. This approach may boost the development of Nox inhibitors as new therapeutic molecules for PD.
Aims: Parkinson’s disease (PD) is the second most common neurodegenerative disorder, characterized by progressive neurodegeneration, extensive accumulation of α-synuclein aggregates and chronic neuroinflammation. We previously developed vinyl sulfone derivative as an Nrf2 activator KDS4043 and demonstrated its therapeutic effects in an MPTP-induced PD mouse model. However, further studies are needed to evaluate KDS4043 as a promising agent for treatment of PD, because several compounds with neuroprotective effects in neurotoxin-based animal models have failed in clinical trials.

Methods: We evaluated the therapeutic effects of Nrf2 activator KDS4043 in the rAAV-A53T PD mouse model by assessing behavioral test and brain tissue analysis. Furthermore, we investigated whether KDS4043 regulates NLRP3 inflammasome involved in chronic inflammation in the rAAV-A53T model and cell lines. We then tested if KDS4043 could modulate NLRP3 inflammasome via autophagy pathway.

Results: Treatment of Nrf2 activator KDS4043 mitigates PD-like motor deficits by protecting dopaminergic neurons and alleviating α-Syn pathology in rAAV-A53T model. Furthermore, NLRP3 inflammasome is attenuated by KDS4043 in rAAV-A53T model. In addition, we observe that Nrf2 activator KDS4043 upregulates autophagy and attenuates activation of NLRP3 inflammasome through P62-mediated autophagy pathway in vitro.

Conclusions: We discover that Nrf2 activator KDS4043 can modulate NLRP3 inflammasome via autophagy pathway and suggest that KDS4043 can be a potential drug candidate for the treatment of PD.
Aims: To design novel small molecule Shc inhibitors and test them in cell and animal models of AD. 

Methods: We tested neuroprotective potency of Shc blockers to rescue N2A cells challenged with A-beta in 384-well plates and also tested the anti-inflammatory potency of Shc blockers in human and mouse microglial cells challenged with pro-inflammatory A-beta. Also, the impact of Shc Blockers on memory loss by Barnes Maze and Novel Object Recognition and LTP was tested in the mouse AD models ApoE4 and PSAPP.

Results: Multiple Shc blockers protected from A-beta-dependent neurotoxicity in N2a cells, and from A-beta-mediated microglial inflammation. We observe rescue of LTP in the PSAPP mouse model of AD by Shc blockers. We observe protection from memory loss in the ApoE4 mouse model of AD in vivo.

Conclusions: Conclusion. These data suggest that small-molecule Shc blockers are a novel therapeutic strategy for AD. Question&Background. Our question is whether small-molecule Shc inhibitors ameliorate AD in cell and animal AD models. The human Shc locus has recently been identified as the 12th most important new locus in AD [PMID: 30944912] and impacts MCI to full Alzheimer's Disease conversion, and is assayable in blood [PMID: 33172501]. Cells depleted of Shc resist A-beta toxicity [PMID: 3045931: PMID: 15837797]. Shc-depleted PSAPP mice resist AD with the same amyloid burden [PMID: 27431297]. Thus Shc reduction is neuroprotective, downstream of the toxic A-beta signal [PMID: 25012499]. Novel molecules invented by Resistomix were tested for their potency to ameliorate AD pathophysiology.
POSTERS

GENETIC ABLATION OF GPNMB DOES NOT ALTER SYNUCLEIN-RELATED PATHOLOGY

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Aims: The gene GPNMB is known to play roles in phagocytosis and tissue repair, and is upregulated in microglia in many mouse models of neurodegenerative disease as well as in human patients. Nearby genomic variants are associated with both elevated Parkinson's disease (PD) risk and higher expression of this gene, suggesting that inhibiting GPNMB activity might be protective in Parkinson's disease.

Methods: We tested this hypothesis in three different mouse models of neurological diseases: a remyelination model and two models of alpha-synuclein pathology.

Results: We found that Gpnmb deletion had no effect on histological, cellular, behavioral, neurochemical or gene expression phenotypes in any of these models.

Conclusions: These data suggest that Gpnmb does not play a major role in the development of pathology or functional defects in these models and that further work is necessary to study its role in the development or progression of Parkinson's disease.
REVERSIBLE MAO-B INHIBITOR, AS AN EFFECTIVE THERAPEUTIC CANDIDATE FOR PARKINSON’S DISEASE

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Aims: Monoamine oxidase-B (MAO-B) is a well-established therapeutic target for Parkinson’s disease (PD); however, previous clinical studies on currently available irreversible MAO-B inhibitors have yielded disappointing neuroprotective effects. Here, we tested the therapeutic potential of reversible MAO-B inhibitor in multiple animal models of PD.

Methods: We designed and synthesized a series of $\alpha$-aminoamide derivatives and evaluated their MAO-B inhibitory potency, specificity, reversibility, and bioavailability (>100%). In several animal models of PD (a mouse MPTP model, 6-hydroxydopamine induction and disruption of the substantia nigra striatal pathway in a mouse model of A53T mutant $\alpha$-synuclein overexpression), we demonstrated the significant neuroprotective and anti-neuroinflammatory efficacy of MAO-B inhibitors. Additionally, we performed pharmacokinetic (PK) and toxicity tests in cynomolgus monkeys.

Results: In this study, we demonstrate that 18j is the most potent and selective MAO-B inhibitor among a large array of $\alpha$-aminoamide derivatives. Moreover, a 3-day treatment with 18j prevented or partially reversed MPTP-induced PD-like pathologies, including nigrostriatal TH loss, astrogliosis, microgliosis, and parkinsonian motor deficits. Further, long-term 18j treatment in 6-OHDA model showed more favorable effect than selegiline, an irreversible MAO-B inhibitor widely prescribed for PD. We also demonstrated a favorable PK profile and low toxicity in non-human primates, validated 18j as a potential therapeutic agent for PD patients.

Conclusions: We have demonstrated that MAO-B is a key molecular target for suppression of neuroinflammation in both PD and AD, suggesting that reversible MAO-B inhibitors with good biosafety and bioavailability are promising candidate treatments for these neurodegenerative disorders. Furthermore, astrogliosis could be an indication for reversible MAO-B inhibitor treatment.
POSTERS

BRAIN-PENETRANT STRUCTURALLY TARGETED ALLOTERIC REGULATORS FOR GLUCOCEREBROSIDASE (GCase) SHOW PROMISING PHARMACOLOGICAL ACTIVITY IN MODELS OF NEURODEGENERATIVE DISEASES

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Aims: Mutations in the GBA1 gene, encoding the lysosomal enzyme GCase, represent the most common genetic risk factor for Parkinson’s disease (PD). Impaired GCase function has earned attention due to its association with α-synuclein pathology in GBA-associated PD patients, but also in sporadic PD, as well as in related α-synucleopathies. Although less investigated, decreased GCase levels and activity is also involved with the pathophysiology of Alzheimer’s disease (AD). Importantly, overexpression of GCase was shown to promote lysosomal degradation of α-synuclein and Amyloid-Beta (Aβ) toxic forms, thus contributing to ameliorate symptoms in PD and AD, respectively. Enhancing the activity of mutant and wild-type GCase may represent a therapeutic strategy for the treatment of neurodegenerative diseases.

Methods: Gain Therapeutics has applied the innovative proprietary drug discovery platform, Site-directed Enzyme Enhancement Therapy (SEE-Tx™), to the development of small-molecule structurally targeted allosteric regulators (STARs) that stabilize misfolded GCase avoiding its degradation whilst facilitating its maturation and trafficking to the lysosomes.

Results: We report in vitro and in vivo evidence showing that by increasing both mutant and wild-type GCase activity, our orally bioavailable and brain penetrant lead STARs reduce neurotoxicity and inflammation derived from the accumulation of toxic oligomeric species of Aβ, tau and α-synuclein in the brain, as well as ameliorate behavioral deficits.

Conclusions: Improvement of lysosomal function through the enhancement of GCase activity and levels can ameliorate symptoms in α-synucleinopathies and AD. This therapeutic approach represents a valid option for the treatment of neurodegenerative diseases, thus warranting further development towards the clinic.
PROGRESS OF CNS AGGREGATED PROTEIN DEGRADATION THERAPEUTICS FROM APRINOIA’S SMALL MOLECULE DISCOVERY PLATFORM


1APRINOIA; Therapeutics, Chemistry, Wanchai, Hong Kong PRC, 2APRINOIA; Therapeutics, Biology, Wanchai, Hong Kong PRC, 3APRINOIA; Therapeutics, Clinical, Wanchai, Hong Kong PRC

Aims: The hallmark of neurodegenerative diseases, such as Parkinson's disease (PD) and Alzheimer's disease (AD), is an accumulation of protein aggregates, which lead to neurotoxicity and dysfunction. Small molecule therapeutics that can remove pathological aggregates are highly desired for both diseases.

Methods: Our small molecule discovery platform consists of our proprietary small molecule collection, unique PET imaging biomarkers and cryoEM structures. Our proprietary collection of CNS-focused aggregated protein binding agents, previously developed by APRINOIA to map the structure-activity relationship of its tau PET tracer programs, is selectively coupled with agents designed to hijack cellular quality-control systems which facilitate aggregation clearance. We have developed a screening funnel to interrogate the ability of our small molecules to affect clearance of protein aggregates.

Results: Therapeutic compounds developed have been shown to bind to intracellular tau aggregates in fluorescence assays. Furthermore, we have identified compounds that show proteosome dependent elimination of aSyn aggregates in the ReNcell VM neuronal model. PK studies show that these compounds can cross the BBB. In vivo studies with rTg4510 mice show that a single dose of our degraders can affect reduction of tau protein.

Conclusions: APRINOIA’s small molecule discovery platform has produced lead compounds capable of degrading protein aggregates in cellular assays and in vivo models. Further development is ongoing.
INVESTIGATION OF AN AAV-BMP2 VIRAL VECTOR ON MOTOR BEHAVIOURS IN A RAT AAV-ASYN MODEL OF PARKINSON’S DISEASE

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Aims: We sought to test the hypothesis that viral delivery of the neurotrophic factor bone morphogenetic 2 (BMP2) to the substantia nigra (SN) would protect dopaminergic neurons from degeneration and motor impairments induced by the overexpression alpha-synuclein (αSyn) in a rat model of Parkinson’s Disease (PD).

Methods: To test this hypothesis, adult male and female Sprague-Dawley rats received a unilateral injection AAV-αSyn to the SN. Four weeks later, they received a unilateral injection AAV-BMP2 or an AAV-Control vector to the same SN. Behavioural testing (stepping and cylinder tests) were carried out at 12, 16, 20 and 24 weeks before the brains were processed for immunohistochemistry to analyse nigrostriatal integrity at 24 weeks.

Results: Longitudinal behavioural testing revealed that 4 weeks after injection of AAV-αSyn there were significant impairments in contralateral paw use compared to the ipsilateral paw in the behavioural tests. This indicated that there was some degree of nigrostriatal damage at the time of AAV-BMP2 delivery. Longitudinal behavioural testing after administration of AAV-BMP2 however revealed that there were no significant improvements in contralateral paw use in male or female rats when they were tested at 12-24 weeks.

Conclusions: BMP2 has previously been shown to have neurotrophic effects on DA neurons in vitro. Moreover it is a member of a neurotrophic factor family whose other member, GDF5, have been shown to have beneficial effects in this model. As such the lack of effect of AAV-BMP2 on behavioural outcomes is somewhat surprising but post-mortem analysis of transgene expression and of nigrostriatal integrity is now required.
Aims: The purpose of this project is to target the SNCA gene encoding for α-synuclein (α-syn), a molecule central to Parkinson’s disease (PD) pathogenesis.

Methods: The CRISPR/Cas9 gene-editing tool allows selective disruption of disease-related alleles as a novel therapeutic approach for CNS disorders. We are adopting this system for targeting the SNCA gene.

Results: This strategy allows us to selectively disrupt 13% of the PD-associated A53T SNCA mutation in patient’s fibroblasts. We were also able to achieve general indel formation in the SNCA gene in order to modulate the level of transcript.

Conclusions: Our results present possible treatment options for the specific mutated site, without targeting the wild type allele. On the other hand partial SNCA disruption aim to suppress the levels of a protein that deposits in the brain and would be a useful therapy also for the vast majority of sporadic disease cases as well as patients harboring SNCA duplications and triplications.
Aims: Multisystem Atrophy (MSA) is a rapidly progressive synucleinopathy with no disease modifying agents available. In MSA, the majority of misfolded alpha synuclein (aSyn) resides in the intracellular space, predominantly in oligodendroglia and in neurons. We have developed a scFv monoclonal antibody derivative (CGX208) effectively binding and engaging low molecular aSyn oligomers as well as preformed fibrils with minimal reactivity to the native protein. Herein, we describe the application of a gene therapy approach, where the scFv was cloned to AAV9 (CGX218) and tested for efficacy in MSA.

Methods: CGX218 was delivered instriatally to rats with an inducible and aggressive experimental MSA model. Animals were tested for sustained expression of the scFv (W.B, ELISA), motoric dysfunction (cylinder test) and dopaminergic nerve loss (Th1 staining).

Results: A single delivery of CGX218 after significant dopaminergic damage has been obtained, was sufficient to result in a robust and sustained expression of the scFv targeting misfolded aSyn in the brains. Importantly, the scFv was predominantly expressed in neurons and oligodendroglia as evident from immunohistochemical studies. A single intracerebral delivery of CGX218 resulted in a significant dose dependent attenuation of motoric dysfunction in the cylinder test that was associated with a robust protection from dopaminergic nerve loss, exhibited by Th1 staining.

Conclusions: Thus, a strategy of driving expression of a scFv targeting misfolded aSyn using an AAV9 vector was highly effective in treatment of experimental MSA even when given after significant neurodegeneration has already occurred.
TRANSLATION OF ALLOPREGNANOLONE REGENERATIVE THERAPEUTIC IN PARKINSON’S DISEASE: A PILOT STUDY FRAMEWORK

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Aims: To develop a pilot study framework to assess the feasibility of a randomized-controlled trial to evaluate the safety, tolerability, and effect of allopregnanolone, a neurosteroid, in patients with Parkinson’s disease (PD).

Methods: We plan to conduct a phase 1b open-label clinical trial of 12 weeks duration to assess the safety, tolerability, and potential effects of allopregnanolone in patients with PD. A total of 12 participants will be recruited, 6 males and 6 females, age 40 – 80 years, with a history of idiopathic sporadic PD, and who are APOE4 genotype positive. Allopregnanolone 4mg will be administered once-per-week via an intravenous infusion of 30-minute duration. Primary objectives are safety and tolerability over 12-weeks. Secondary objectives are to evaluate potential indicators of efficacy on motor and non-motor symptoms (cognition and sleep disturbances) over 12 weeks.

Results: Previous preclinical data demonstrated that treatment with allopregnanolone once-per-week for 2-weeks restored the number of tyrosine hydroxylase immunoreactive neurons and total cell counts in in the nigrostriatal tract of MPTP lesioned mice and increased BrdU-positive cells in the substantia nigra pars compacta¹. In mouse model of Alzheimer’s disease, it induced neurogenesis correlated with restoration of learning and memory function², and reduced microglial activation³. Clinical data of allopregnanolone in other neurodegenerative diseases has demonstrated safety, tolerability, and feasibility for evaluation of efficacy indicators⁴,⁵.

Conclusions: Data from preclinical, translational, and clinical research support advancing allopregnanolone as a therapeutic to address symptoms of PD and as a therapeutic to potentially modify the course of disease progression.
POSTERS

NONINVASIVE BRAINSTEM MODULATION FOR THE TREATMENT OF NONMOTOR SYMPTOMS IN PARKINSON’S DISEASE: RANDOMIZED CONTROLLED TRIAL AND OPEN LABEL EXTENSION STUDY.

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Aims: A multi-site double-blinded randomized clinical trial (RCT; NCT04797611) and open label extension study (OLE; NCT04799418) have been initiated to evaluate the safety and efficacy of time-varying caloric vestibular stimulation (tvCVS), using the ThermoNeuroModulation (TNM™) Device, developed by Scion NeuroStim, LLC, for the treatment of Parkinson’s Disease (PD). Primary: Evaluate the safety and efficacy of tvCVS for reducing non-motor symptom (NMS) burden in PD. Secondary: Establish whether tvCVS improves activities of daily living related to motor function, provides clinically meaningful benefits, improves motor symptoms and improves the quality of life for participants diagnosed with PD. Exploratory: Evaluate the impact of tvCVS on individual NMS, explore the temporal kinetics of motor symptom response to treatment and evaluate the potential of tvCVS to improve gait.

Methods: 218 PD patients taking stable doses of oral anti-Parkinsonian medications will be randomized (1:1) to self-administer either tvCVS or passive treatment twice-daily in the home setting over 12 weeks. Participants will then enter the OLE during which all will self-administer twice-daily tvCVS treatment for 12 weeks. Study participants will be followed for 16 weeks post-treatment-cessation, then twice-daily treatments will be re-introduced for 8 weeks.

Results: Data collection is currently ongoing; however, in a previous single site RCT, tvCVS treatments were associated with robust and clinically-meaningful improvements across a broad spectrum of PD symptoms with an absence of significant risk to balance, gait, or safety.

Conclusions: These studies will provide critical data to evaluate tvCVS as a safe and effective adjuvant treatment for addressing multiple unmet needs for PD patients.
MULTIMODAL EFFECTS OF SYSTEMIC PACAP GLYCOPEPTIDE DELIVERY IN RODENT MODELS OF PARKINSON’S DISEASE

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Aims: We are testing the ability of the glycosylated neurohormone Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) to induce neuroprotection and/or restoration in two rodent models of Parkinson’s disease (PD). The glycosylation at peptidase sites successfully improves peptide half-life and blood-brain barrier penetration. In order to determine if systemic administration of PACAP₁⁻²⁷S-Lac is able to reduce non-motor (cognitive and other) symptoms, clear toxic alpha-synuclein aggregates and reduce inflammation, we are currently assessing its effects in human alpha-synuclein overexpressing (Thy1-αSyn) mice.

Methods: Thy1-αSyn or wild-type mice were treated with 1 mg/kg PACAP₁⁻²⁷S-Lac (SC, alternate days) or saline starting at 2 months of age (n= 7-12/group). After one month, mice were subjected to a range of motor and non-motor (cognitive and other) behavioral tasks, and sacrificed for blood biochemistry (inflammatory cytokines) and immunohistochemical analysis (αSyn, phospho-αSyn and glial markers such as Iba1, CD68, GFAP).

Results: Preliminary results are encouraging and suggest that Thy1-αSyn mice treated with PACAP₁⁻²⁷S-Lac display a 31% reduction in coordination and gait disturbances as evaluated by a tapered beam task. Immunohistochemical analysis of the αSyn pathology and glial reactivity, as well evaluation of inflammatory cytokines in the blood is ongoing.

Conclusions: Prior unpublished results indicate that PACAP₁⁻²⁷S-Lac can protect against dopaminergic (DA) cell loss in a mild progressive 6-Hydroxydopamine (6-OHDA) rat model. Together these data indicate that PACAP₁⁻²⁷S-Lac (1) promotes DA neuron survival and (2) shows potential to improve both motor and non-motor outcomes in the Thy1-αSyn mice. Ongoing studies will further clarify the therapeutic potential of PACAP₁⁻²⁷S-Lac to address PD.
METHODOLOGY FOR THE DEVELOPMENT OF TARGETED DRUG DELIVERY TO DOPAMINERGIC NEURONS AS A WAY TO IMPROVE THERAPY IN PARKINSON'S DISEASE

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Aims: This study aimed to develop a drug delivery methodology to nigrostriatal dopaminergic neurons that will improve the current treatment of Parkinson's disease (PD). This methodology is based on the idea about the possibility of specific internalization of substances with a high affinity for the dopamine transporter (DAT).

Methods: As a model substance, we used 1- (2- [bis (4-fluorophenyl) methoxy] ethyl) -4- (3-phenylpropyl) piperazine (GBR) -BODIPY (BP), an original fluorescent analogue of tDAT ligand GBR-12909. The GBR-BP internalization was tested in primary cultures of the mouse embryonic mesencephalon and metencephalon, and in immortalized cells of human embryonic mesencephalon (LUHMES). GBR-BP-stained cells were fixed and immunostained for tyrosine hydroxylase, dopamine beta-hydroxylase and serotonin. In the control, the above cells were pretreated with GBR-12909, an inhibitor of monoamines, mainly dopamine uptake.

Results: Using a primary culture of the mouse mesencephalon and LUHMES cells, it was proven that GBR-BP is internalized into dopaminergic neurons due to a DAT-dependent mechanism. Moreover, in primary metencephalon culture GBR-BP is also internalized into noradrenergic and serotoninergic neurons via membrane transporters. For the future use of this approach to the development of neuroprotective therapy for PD, our data proving that GBR-BP does not have a toxic effect on dopaminergic neurons in mice and humans are of particular importance.

Conclusions: Thus, our data open up a wide prospect for the continuation of the development of targeted drug delivery to dopaminergic neurons, and for visualization of living dopaminergic neurons in a mixed population of brain neurons. This work was supported by № 13.1902.21.0027 / RF-19022X0027.
CHRONIC EXERCISE REDUCES SYNUCLEINOPATHIES BY MODULATING OXIDATIVE STRESS RESPONSES IN PARKINSON’S DISEASE

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Aims: Non-pharmacological exercise therapy has been consistently proposed for Parkinson's disease, but its value in altering the progression of disease is unknown. In particular, the mechanisms of exercise in spread of α-synuclein aggregates remain undefined. In this study, we presented evidence for improvement of synucleinopathies and behavioral deficits after chronic exercise in α-synuclein transgenic models, and demonstrated the mechanisms of degenerative regulation by analyzing candidate genes.

Methods: Five months old α-synuclein TG were treated with a voluntary running wheel for 3 months. We examined α-synuclein deposition, neurodegeneration and behavioral deficits, as measured by immunohistochemistry, motor and cognitive behavior tests. Based on the results of behavioral changes, we also performed RNAseq by extracting RNA from brain regions.

Results: Alpha-synuclein TG treated with voluntary exercise restored motor deficits, decreased α-synuclein deposition, neurodegeneration and neuroinflammation in cortex and HP, and increased dopaminergic terminal in striatum region. The results of RNAseq in motor cortex regions represent a molecular mechanism for altering synucleinopathies and neurodegeneration. Analysis of RNAseq data by gene ontology showed that the mechanisms involved mitochondria function and ER associated protein degradation have changed significantly, which regulate oxidative stress response.

Conclusions: These results suggest that chronic exercise modifies the mechanism of oxidative stress response genes in the midbrain and prevents synucleinopathies and motor disfunction. Therefore, exercise as therapeutic strategy could slow down the progression of synuclein aggregates and other neurodegenerative diseases.
INVESTIGATING THE CONTRIBUTION OF THE PERIAQUEDUCTAL GREY IN PAIN IN PARKINSON’S DISEASE

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Aims: Pain is one of the most debilitating non-motor symptoms seen in Parkinson’s disease (PD). The aetiology of PD-related pain is elusive and conventional analgesics remain inadequate. We hypothesised that pathology in the periaqueductal grey (PAG) contributes to pain in PD by altering opioid tone in the spinal cord. We examined this using the 6-hydroxydopamine (6-OHDA)-lesioned rat model of PD which exhibits reduced nociceptive thresholds.

Methods: Thermal and mechanical hypersensitivity were assessed before and after 6-OHDA injection into the medial forebrain bundle (MFB; PD model) or PAG of male Wistar rats. Post-mortem, immunohistochemistry was performed in the PAG and spinal cord. Separately, we examined the ability of a dopamine D1 agonist injected directly into the PAG to reverse 6-OHDA-induced mechanical hypersensitivity in the PD model.

Results: Rats with MFB 6-OHDA lesions exhibited reduced thermal and mechanical thresholds in both hind paws. Met-enkephalin was reduced bilaterally in the dorsal horn of the spinal cord, while ipsilateral reductions of dopaminergic cells were seen in the PAG. When 6-OHDA was used to directly ablate dopaminergic cells in the PAG, similar reductions in nociceptive thresholds and met-enkephalin levels were seen. Injection of the D1-like agonist, SKF38393 (1 microgram/0.5 microlitre), into the PAG of parkinsonian rats reversed this mechanical hypersensitivity.

Conclusions: 6-OHDA lesioned rats exhibit hypersensitivity that may result from a reduction in spinal cord opioid tone secondary to dopaminergic cell loss in the PAG. This hypersensitivity can be reversed by SKF38393, highlighting D1-like receptors in the PAG as a novel analgesic target in PD.
THE NEUROPROTECTIVE ROLE OF GM1-OLIGOSACCHARIDE IN PARKINSON’S DISEASE

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Aims: GM1 ganglioside has been considered as a master regulator of the nervous system and accumulating evidence is pointing out age-dependent GM1 deficiency as initiator of sporadic Parkinson’s disease (PD). Preclinical data have reported that GM1 administration exerts neuroprotective and neurorestorative properties, although the benefit resulting from GM1 replacement therapy is extremely limited by its amphiphilicity that prevents the BBB passage. Recently, we demonstrated in neuronal cells that the oligosaccharide portion of GM1 (OligoGM1) is the actual moiety responsible for GM1 neurotrophic properties. Thus, in this scenario we decided to evaluate the OligoGM1 neuroprotective potential.

Methods: We tested the OligoGM1 in two in vitro PD models: i) DA neurons intoxicated with MPTP, a toxin that affects mitochondria, causing ROS over production and ii) α-synuclein oligomers (aSynO) injured DA neurons reproducing the essential neuropathological features of PD. Moreover, OligoGM1 was administered to B4galnt1−/− mice and to MPTP mouse, both in vivo models of sporadic PD.

Results: By biochemical analysis, we observed that the pre-treatment of DA neurons with OligoGM1 significantly increases neuronal survival and preserves neurite networks affected by aSynO and by MPTP. In vivo, we found that following MPTP administration in mice, OligoGM1 restores nigral tyrosine hydroxylase expression reaching the healthy condition. Moreover, OligoGM1 administered to B4galnt1−/− mice reduces nigral αS aggregates and restore DA tyrosine hydroxylase neurons.

Conclusions: The obtained data suggest that OligoGM1 administration protects DA neurons probably triggering a trophic signal starting at plasma membrane and implementing mitochondrial bioenergetics, reducing oxidative stress and augmenting the α-synuclein clearance.
ANAVERSE®2-73-ANALYSIS OF MOVEMENT (MDS-UPDRS) AND COGNITIVE (CDR SYSTEM) PHARMACODYNAMIC-BIOMARKER OUTCOME MEASURES PLACEBO-CONTROLLED PHASE 2 TRIAL IN 132 PARKINSON’S DISEASE DEMENTIA PATIENTS

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Aims: ANAVERSE®2-73 (blarcamesine), a novel, oral, investigational SIGMAR1 was assessed for movement and cognition in patients with Parkinson’s disease dementia (PDD) measuring motor and non-motor complications (MDS-UPDRS) and cognition (CDR system) and pharmacodynamic-biomarker analysis including mRNA SIGMAR1.

Methods: ANAVERSE®2-73-PDD-001 14-week study was an international, double-blind, multicenter, placebo-controlled Phase 2 clinical study. 132 patients with PDD were randomized equally to target doses of 30mg, 50mg ANAVERSE®2-73 or placebo. MDS-UPDRS, which is a validated global test for PD was assessed to evaluate various aspects of motor and non-motor complications, while cognition was evaluated with Cognitive Drug Research computerized assessment (CDR) system, which is an automated test battery validated for use in AD, PDD and other dementias.

Results: MDS-UPDRS Total score improved significantly by -14.51 (p=0.034) for patients treated with ANAVERSE®2-73 high oral dose once-daily compared to placebo. The improvement is clinically relevant corresponding to a relative improvement of 18.9% over 14 weeks. Balanced and global improvements were observed: All MDS-UPDRS sub-scores Part I-IV improved with vast majority of individual items between >71% and >92%. SIGMAR1 mRNA expression significantly increased in ANAVERSE®2-73-treated patients vs placebo (p=0.035) and significantly associated with improvements of MDS-UPDRS scores and cognitive efficacy endpoints CDR system.

Conclusions: ANAVERSE®2-73 was generally safe, well tolerated and demonstrated dose-dependent efficacy for both motor impairment (MDS-UPDRS) and cognition (CDR system), which correlated with SIGMAR1 mRNA as a pharmacodynamic biomarker, respectively. These results support continued development of ANAVERSE®2-73 in PD and PDD as well as currently ongoing Precision Medicine biomarker-driven late-stage clinical studies in Rett syndrome and Alzheimer’s disease.
A BRAIN-DELIVERABLE PARKIN PROTECTS NEURONS BY SUPPRESSING ACCUMULATION OF DAMAGED MITOCHONDRIA AND PATHOLOGICAL α-SYNUCLEIN

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Aims: Parkinson’s disease (PD) is a neurodegenerative disease that results in abnormal motor functions due to the selective loss of dopaminergic neurons with mitochondrial dysfunction and formation of pathologically modified α-Synuclein in the brain. Parkin is an E3 ubiquitin ligase that plays a critical role in the replacement of damaged mitochondria and seems to interact with a modified form of α-Synuclein. In the current study, improved cell-permeable (iCP) Parkin containing an advanced macromolecule transduction domain (aMTD), a hydrophobic cell-penetrating peptide, has been developed as an anti-PD therapeutic agent, demonstrating its brain delivery and neuroprotectivity.

Methods: In vitro and in vivo models induced by 6-OHDA were treated with iCP-Parkin.

Results: iCP-Parkin following the successful optimization of purification process for mass production, also maintains auto-ubiquitination functionality and shows neuro-protective activities against oxidative stress. As a neuroprotective action mode, iCP-Parkin is shown to promote mitophagy as well as induce expression of mitochondrial biogenesis factors, replacing damaged mitochondria. In addition, iCP-Parkin suppresses the accumulation of pathogenic α-Synuclein in α-Synuclein-overexpressing cells. In 6-hydroxydopamine (6-OHDA)-induced PD mice, iCP-Parkin recovers motor functions as assessed by rotarod (52%) test, and increases the expression (90%) of tyrosine hydroxylase (TH) in dopaminergic (DA) neurons. Lastly, BBB-permeability of iCP-Parkin has been measured by LC-MS/MS analysis to explore the aMTD-mediated brain delivery. iCP-Parkin is detected in the brain, but Non-CP-Parkin lacking aMTD sequence is not.

Conclusions: Based on these outcomes, iCP-Parkin has a great therapeutic potential as a PD-modifying agent with superior productivity.
SAFETY, TOLERABILITY, AND PHARMACOKINETICS OF THE OLIGOMER MODULATOR ANLE138B: A FIRST-IN-HUMAN RANDOMISED, DOUBLE-BLIND, PLACEBO-CONTROLLED PHASE 1 TRIAL

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Aims: Synucleinopathies such as Parkinson’s disease, Dementia with Lewy bodies and Multiple System Atrophy are characterized by deposition of misfolded and aggregated α-synuclein. Small aggregates (oligomers) of α-synuclein have been shown to be the most relevant neurotoxic species and are targeted by anle138b, an orally bioavailable small molecule compound which shows strong disease-modifying effects in animal models of synucleinopathies. Here we present first-in-human data of anle138b and compare exposure with pharmacokinetics/pharmacodynamics data in animals.

Methods: Anle138b was studied in a single-centre, double-blind, randomised, placebo-controlled single ascending dose and multiple ascending dose study in healthy subjects. Participants were randomly assigned to placebo or anle138b (dose range 50 - 300 mg per day), respectively. The primary endpoints were safety and tolerability, the secondary endpoint was pharmacokinetics. In addition, the effect of food on the pharmacokinetics of anle138b was examined. Treatment was administered orally in hard gelatine capsules containing either anle138b or excipient only. Clinicaltrials.gov-identifier: NCT04208152. EudraCT-number: 2019-004218-33.

Results: 196 healthy volunteers were screened and 68 participants were enrolled. Of these, all completed the study per protocol. There were no major protocol violations. Anle138b demonstrated excellent safety and tolerability at all dose levels. No abnormal trend was seen in any system organ class. Already at multiple doses of 200 mg, exposure levels above the fully effective exposure in the MI2 mouse Parkinson model were observed.

Conclusions: Anle138b was safe and well tolerated in doses resulting in exposure levels above those fully effective in a mouse Parkinson model. These findings warrant further clinical trials in patients with synucleinopathies.
Aims: Synucleinopathies, including Parkinson’s disease, are lethal neurodegenerative diseases that are characterised by the formation of amyloid fibrils of the protein \( \alpha \)-synuclein (\( \alpha \)-syn) in the brain. Amyloids are formed from soluble, usually monomeric, proteins that, in disease, misfold and self-assemble into fibrils. These fibrils are composed of protofilaments with a common cross-\( \beta \) structure. Multiple different conformations, including monomers, tetramers, higher-level oligomers and fibrils can be formed by \( \alpha \)-syn, which can form seeds and self-propagate in a prion-like manner. Antibodies are established biologics and important research tools. Single-domain antibodies are advantageous due to their simple, robust scaffold and small size that allows them to reach buried protein cavities. Our goal is to discover single-domain antibodies that bind to \( \alpha \)-syn and inhibit its aggregation.

Methods: To achieve our goal, ribosome display is used, coupled with a high-throughput screening method for the identification of hits. This method involves selection of the desired antibodies using periplasmic extracts of Escherichia coli overexpressing the antibody candidates.

Results: Our results show that we have discovered a number of antibodies that bind to \( \alpha \)-syn and inhibit its aggregation. The antibodies are being characterised using various methods, including circular dichroism and surface plasmon resonance.

Conclusions: More importantly, the antibodies inhibit \( \alpha \)-syn aggregation at substoichiometric concentration, using a molar ratio of 1 to 10, that suggests preferential binding of the discovered antibodies to the aggregating intermediates.
POSTERS

THE TETRACYCLINE-CLASS ANTIBIOTIC DOXYCYCLINE PROTECTS DOPAMINE NEURONS FROM FERROPTOTIC CELL DEATH

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Aims: Doxycycline (DOX) an old tetracycline (TC) antibiotic primarily used for skin problems recently proved to be effective in model systems that relate to Parkinson disease (PD) neurodegeneration (Dominguez-Mejide et al, Neurobiol Dis, 2021; Perry et al, Nat Metab, 2021; Ferreira Jr et al, Cells, 2021). The present work aimed at better characterizing the mechanisms underlying DOX-mediated neuroprotection.

Methods: For that, we used a model system of mouse primary midbrain cultures, in which the progressive loss of dopamine (DA) neurons results from ferroptotic cell death induced by the presence of catalytic iron in the culture medium.

Results: We found that DOX provides concentration-dependent protective effects for DA neurons with an EC50 of about 4.8µM. Rescued neurons were functional as they accumulated tritiated-DA efficiently. Live imaging of reactive oxygen species with DHR-123 revealed that DOX efficiently repressed intracellular oxidative stress, presumably of mitochondrial origin. The iron chelating agent desferrioxamine, the hydrogen peroxide scavenger enzyme catalase, the inhibitor of lipid peroxidation Trolox and the ferroptosis inhibitor liproxstatin-1 mimicked the neuroprotective and antioxidant effects of DOX, indicating that DOX neutralized the effects of an iron-mediated Fenton reaction promoting lipid peroxidation. Interestingly, the effects of DOX persisted in aged cultures that may model more closely PD neurodegeneration. Noticeably, non-TC antibiotics such as streptomycin, erythromycin and penicillin-G did not reproduce DOX protective effects.

Conclusions: Overall, our data indicate that DOX has the potential to interfere efficiently with ferroptotic cell death that relates more specifically to PD neurodegeneration. Supported by France Parkinson (DOXYPARK, GAO 2018).
Aims: There are no evidence-based disease modifying interventions for DLB and the underlying pathophysiology is not yet fully understood. However, evidence suggests that reduced glucocerebrosidase activity is involved, and ambroxol is a drug that increases glucocerebrosidase activity and protein levels. As CNS penetration is confirmed, repurposing use has been suggested for PD/DLB. In fact, ambroxol proved promising in slowing disease progression in PD; therefore, the objective of this study is to investigate the effects on DLB.

Methods: The ANeED study is a national multicenter phase IIa RCT clinical intervention study including patients with prodromal and mild DLB with a MMSE >14. The drug intervention is 420 mg. ambroxol or placebo three times daily. Stratification into the treatment or control group is based on APOE e4 genotypes and amyloid-beta CSF concentration. Allocation ratio 1:1 for ambroxol and placebo.

Results: As of October 2021, six out of seven sites have started, the seventh site plan study start within early 2022. 15 participants have been included. Drug compliance is satisfactory (>90%) in 100%. The adverse events reported thus far are falls in 33% and nausea in 25% of the participants. Data will be presented in tables. The plan is to include 172 participants in total.

Conclusions: The ANeED study is recruiting according to plan, and we expect an accelerated recruitment during the next six months. Thus far, the tolerability of ambroxol is satisfactory.
Aims: ND0612 is in development as the first continuous subcutaneous (SC) levodopa/carbidopa delivery system for patients with Parkinson’s disease (PD) and motor fluctuations. A population PK model of levodopa and carbidopa following subcutaneous infusion of ND0612 with/without oral therapy was developed.

Methods: Two integrated population PK models (for levodopa and for carbidopa) were developed using data from two phase-1 studies of ND0612 (Studies 004 and 005) in PD patients and healthy volunteers, respectively. The predictive performance of each model was then tested using data from a third phase 1 study in healthy volunteers (Study 114). Model refinement was performed using aggregated data from the three studies and will be updated as sparse PK data from ongoing studies becomes available.

Results: Levodopa and carbidopa population PK models were both adequately described by a one compartment model with sequential zero and first-order SC absorption and first-order oral absorption (Figure). Carbidopa had linear elimination from the central compartment. Levodopa had parallel dopa decarboxylase (DDC) and catechol-O-methyltransferase (COMT) elimination from the central compartment, in which the inhibition of apparent DDC-mediated clearance was driven by carbidopa plasma concentrations. Exploration of covariates showed age had a significant effect on apparent clearance and apparent volume of distribution for both carbidopa and levodopa, even after accounting for body weight differences; both parameters decreased with increasing age.

Conclusions: Model diagnostics for the carbidopa and levodopa population PK models indicated a satisfactory predictive performance, supporting their usability to derive individual predictions of exposure to be used in future pharmacokinetic-pharmacodynamic analyses.
Aims: To estimate the meaningful within-patient worsening threshold (MWPWT) of the Movement Disorders Society–Unified Parkinson’s Disease Rating Scale (MDS-UPDRS) Part III in early Parkinson’s disease (ePD).

Methods: Data were used (N=316; OFF state for MDS-UPDRS Part III) from the Phase II PASADENA study (NCT03100149). The Clinical Global Impression-Improvement (CGI-I) was selected as the anchor. Spearman correlations between change from baseline scores for MDS-UPDRS Part III and CGI-I were conducted at Weeks 24 and 52. Empirical cumulative distribution function (eCDF) curves for change in MDS-UPDRS Part III scores were plotted for each of the CGI-I response categories at Weeks 24 and 52. To estimate the MWPWT, the mean and median MDS-UPDRS Part III scores were calculated using data from the first visit at which individuals were rated as ‘Minimally worse’ on the CGI-I.

Results: Correlations of 0.31–0.32, and separation of eCDF curves were identified, supporting the use of CGI-I as an anchor. Overall, 251 patients had a CGI-I rating of ‘Minimally worse’ at a post-baseline visit, for whom a mean (median) value of 4.98 (5.00) points on MDS-UPDRS Part III was identified. This supports a MWPWT of 5 points.

Conclusions: These findings indicate that a 5-point increase on MDS-UPDRS Part III can be used as a threshold for meaningful worsening of motor signs. This supports the use of time-to-5-point increase on MDS-UPDRS Part III as the primary endpoint for the Phase IIb PADOVA study, an ongoing randomised, double-blind, placebo-controlled study evaluating the efficacy of prasinezumab in ePD (NCT04777331).
CLINICAL TRIAL PROTOCOL: TRANSCEND 1 – AN OBSERVATIONAL STUDY OF PATIENTS WITH MODERATE PARKINSON’S DISEASE

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Aims: Novo Nordisk is developing a cell therapy for Parkinson’s disease in collaboration with groups at Lund University (Sweden) and the University of Cambridge (UK). The therapy comprises dopaminergic progenitor cells derived from human embryonic stem cells; the clinical development programme currently includes this observational study (TRANSCEND 1) and a planned phase 1/2, proof-of-concept transplant trial (TRANSCEND 2). An observational study ahead of the proof-of-concept transplant trial may reduce some of the confounding factors associated with investigating therapies in Parkinson’s disease. TRANSCEND 1 will follow a cohort of patients with Parkinson’s disease receiving local standard of care (SoC) to monitor fluctuations in individual performance and disease characteristics over time and to facilitate a reliable baseline of disease severity before a subsequent proof-of-concept trial with a novel stem-cell based treatment in some patients from this cohort.

Methods: Patients (n=96) with moderate Parkinson’s disease (50–68 years old), with fluctuating disease and a disease duration of >5 years, will be followed for up to 24 months. They will be examined every 3 months using standard motor (MDS-UPDRS III), cognitive (MoCA), psychiatric (MADRS) and other clinical measures. Patients will receive SoC which may lead to changes in their medication regimen. However, the participants are required to have ≥2 rounds of assessments and 3 months of observation without medication adjustments prior to being invited to be screened for participation in the proof-of-concept transplant trial.

Results: The study will start recruiting in January 2022.

Conclusions: Results are expected in 2024.
Aims: Background: Patient and public involvement/engagement (PPIE) fosters an active partnership between the public and researchers. Involving stakeholders with lived experience also helps ensure meaningful research outputs. To date, PPIE involvement in Lewy body dementia (LBD) research has been limited. Objective: To develop and implement an international PPIE group (UK and Australia) to support the ‘Combining memantine and cholinesterase inhibitors in LBD treatment’ (COBALT) clinical trial.

Methods: The PPIE group (UK; Australia) comprises 23 members, nine with lived experience of LBD and 14 with supporter experience. Members co-produced the terms of reference and a work-plan. They partner with the COBALT researchers to plan and implement the trial, support recruitment, and communicate findings. Activities occur remotely, allowing national and international participation. An evaluation framework was developed to assess impact.

Results: Outputs: The group (1) informed COVID-19-related study protocol amendments; (2) reviewed study documentation for accessibility; (3) contributed to accessible website design; (4) provided critical views on the acceptability of remote consent procedures; (5) inputted on data protection issues. Remote working was demonstrated as feasible and valued by PPIE members. Impact: PPIE input has resulted in important changes to the study protocol and conduct. Empowerment of group members has also led to expansion of activities beyond COBALT by: (1) co-founding a civic organisation, LBD Australia; (2) blogging about living with LBD; (3) participating in Lewy Body Ireland, a new support group; and (4) becoming a Lewy Body Society (UK) trustee.

Conclusions: Embedding LBD-relevant PPIE is feasible and impacts positively on clinical research.
Aims: The objective is to review in the literature evidence of the relationship between the Self, the insular cortex, and Dementia with Lewy bodies (DLB). The notion of “Self” encompasses a subjective part that integrates the most internal bodily phenomena of self-awareness, and an objective part that sustains autobiographical memory and its most specific details. Modifications of the Self, such as anosognosia and personal identity, or autobiographical memory deterioration, are frequently reported by relatives and noticed by clinicians, jointly with insular atrophy, which occurs early in the course of the disease.

Methods: We performed researches on PubMed and Google Scholar, using MeSH subheadings and keywords, limited to papers in English; 226 publications contributed to the review.

Results: The evidence suggest that insula is a multiconnected brain region involved in processing varied aspects of the self. In addition, changes in personal tastes resulting from insular atrophy have been highlighted in DLB. However, there is, to date, little work exploring the different components of the self (i.e. subjective sense of self; self-concept; autobiographical memory) in DLB.

Conclusions: Based on our literature review, it seems that no experimental work have ever been performed on the Self in DLB. Numerous aspects related to the Self, from the most primitive to more elaborated ones are worthy of being studied in this affection, whose anatomic peculiarity sets in early insular damage. We intend to investigate these questionings in DLB, compared to Alzheimer disease and healthy elderly population, by combining behavioral measures and multimodal neuroimaging, with a particular interest for the insular cortex.
POSTERS

NETWORK CONNECTIVITY SHAPES PROGRESSION OF CORTICAL ATROPHY IN PARKINSON'S DISEASE

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Aims: It is hypothesized that neurodegenerative diseases such as Parkinson's disease (PD) are caused by cell-to-cell propagation of misfolded proteins. Evidence for this disease spread through the brain's connectome largely comes from animal experiments whereas translational findings in humans remain limited. We aimed to map the progression of cortical thinning in PD and to test whether this pattern was constrained by network connectivity in the brain.

Methods: T1-weighted MRI scans of de novo PD patients at baseline, 1-, 2-, and 4-year follow-up and healthy controls at baseline were acquired from the Parkinson Progression Marker Initiative (PPMI). For each patient scan, we computed W-score maps to control for effects of normal aging, sex, and scanner site. We modeled longitudinal changes in cortical thickness across follow-up visits for the whole-brain as well as parcel-wise. To test the network spreading hypothesis, we examined the relationship between the atrophy progression in each brain region with the mean deformation across its structurally and functionally connected neighbors.

Results: Mean whole-brain cortical thickness significantly decreased from baseline to 4-year follow-up. Parcel-wise analyses revealed regional atrophy in parietal, inferior temporal, and superior frontal cortex. We also found a significant positive correlation between regional deformation and neighborhood deformation. In other words, the degree of atrophy progression within a given brain region was associated with the collective atrophy of its structurally and functional connected neighbors.

Conclusions: Our results demonstrate a progression of cortical atrophy in early PD that is shaped by connectivity in the brain, lending support to a network spreading model of PD pathology.
ANATOMO-RADIOLOGICAL CORRELATIONS IN A PARKINSON’S DISEASE ANIMAL MODEL.

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**Aims:** Advanced methods in neuroimaging analysis such as radiomics are providing new insights into the mechanisms underlying Parkinson’s Disease (PD). They have shown some predictive abilities in detecting early changes in the brain, and correlating them with different disease related symptoms. By using a preclinical model of the disease, this work aims to decipher the tissular signature of these imaging and texture feature changes by establishing correlation with histological findings.

**Methods:** Sprague Dawley rats receive a double bilateral intranigral injection of AAV-alpha synuclein, inducing PD-like neurodegeneration, then undergo several behavioral tests to evaluate motor and cognitive functions over a 4-month period. MRI acquisitions are held at 2-, 10- and 18-weeks post-injection. They include a whole-brain T2w, T2*w, and MRS in the prefrontal cortex. Histological studies are led to evaluate dopaminergic degeneration, inflammation, iron accumulation and alpha synuclein deposits in the brain.

**Results:** This PD model shows a progressive dopaminergic neurodegeneration and diffuse alpha-synucleinopathy. Deficits in sensori-motricity, visuo-spatial learning and memory, as well as attention are observed. 3D reconstruction of the histological acquisitions allows a precise spatial registration with MRI data. Hence, morphological T2w and quantitative T2*w mapping are expected to spatially and functionally correlate with histological findings, and the model’s cognitive phenotype.

**Conclusions:** Performing imaging analysis on established animal models of PD, and correlating them with the behavioral phenotype and the histological profile has the potential to explain the changes observed in imaging on a cellular and molecular level, and to help better understand the physiopathology of the disease.
LARGER VENTRICLE MAY PREDICT POOR RESPONSE IN THE TREATMENT OF PARKINSON’S DISEASE

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Aims: To explore the impact of cerebral ventricular metrics on treatment response of Parkinson’s disease (PD).

Methods: The current registry-based retrospective cohort study enrolled 101 drug naïve and de novo patients with PD, whom were stratified by the level of Unified Parkinson’s Disease Rating Scale (UPDRS) improvement in 1 year, following as a group of the Best (n = 31; UPDRS III, > 10), the Moderate (n = 43; UPDRS III, 5-10), and the Modest (n = 27; UPDRS III, < 5). The parameters of ventricular metrics include Evan’s index (EI), frontal-occipital horn ratio (FOHR), callosal angle (CA), callosal height (CH), and temporal horn width (THW).

Results: The Best group demonstrated significantly the least metrics in ventricular dimension including EI (vs the Moderate vs the Modest; 0.23 ± 0.02 vs 0.24 ± 0.02 vs 0.26 ± 0.03; p, < 0.001), FOHR (0.64 ± 0.05 vs 0.65 ± 0.05 vs 0.68 ± 0.05; p, 0.025), CA (111.12 ± 9.99 vs 112.43 ± 9.73 vs 98.14 ± 11.27; p, < 0.001), and CH (22.02 ± 5.94 vs 22.05 ± 5.48 vs 25.89 ± 9.32; p, 0.048). Better improvement of the UPDRS III score were observed in patients with smaller ventricular metrics apiece (EI, correlation coefficient (r), 0.637; FOHR, r, 0.405; CA, r, -0.437; CH, r, 0.366). Furthermore, every ventricular parameters were found to predict effectively the treatment response in patients with PD.

Conclusions: Our data demonstrated a negative effect of enlarged ventricle on the treatment of PD patients who were void of evident ventriculomegaly.
POSTERS

ARE DISRUPTIONS IN DYNAMIC FUNCTIONAL CONNECTIVITY CAUSED BY STRIATAL DOPAMINE DEFICIENCY IN PARKINSON’S DISEASE?

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Aims: Recent studies reported disruptions in dynamic functional connectivity (DFC) in patients with Parkinson’s disease (PD). Here, we assessed whether striatal dopamine synthesis capacity modulates DFC states in PD.

Methods: Resting-state fMRI (rs-fMRI) of 59 PD patients and 28 age- and sex-matched healthy controls (HC) were used for analysis. For 43 patients and 13 HC F-Dopa PET scans were available. Rs-fMRI data was pre-processed using CONN (conn-toolbox.org). The striatal synthesis capacity of F-DOPA PET was extracted using the Patlak method. On rs-fMRI data, reduction was conducted using independent component analysis in GIFT. The resulting components were subsequently grouped into 14 resting-state networks. Afterwards, a sliding window approach and k-means clustering was performed on the data to derive DFC states over the whole group. Measures of DFC, such as mean dwell time (i.e. mean time spent in state) and factional time (i.e. total # of windows spent in state) were compared between groups and further correlated with striatal dopamine binding capacity.

Results: The DFC analysis resulted in 4 distinct states, which were characterized by distinct connectivity patterns between the ICs. Although the two groups did not show significant differences in dwell or fractional time, the number of PD patients visiting state 2 at least once was significantly higher. Moreover, greater caudate dopamine synthesis was associated with lower mean dwell and fractional time in states two and one (all p<=0.03).

Conclusions: Our study suggests that the altered temporal properties of functional connectivity observed in PD are potentially influenced by dopamine availability in striatal structures.
IS “OBLITERATION OF DOPAMINE TRANSPORTER UPTAKE” AN IMAGING BIOMARKER OF FBXO7 MUTATION?

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Aims: We report a patient with novel FBXO7 mutation with unique clinical presentation. We summarize updated clinical characteristics of the mutation, and suggest the “obliteration of dopamine transporter uptake” as a possible functional neuroimaging biomarker for FBXO7 mutation.

Methods: This study is a case report.

Results: A 43-year-old male patient presented with a gait disturbance that had progressed 10 months before the first visit. The patient grew up without any problems after having 5 to 6 seizure events before 2 years of age. Ten months before the visit to our hospital, he experienced generalized tonic clonic seizure for about 20 minutes for which he was sent to an emergency room of another hospital and was admitted for treatment of status epilepticus. At the next outpatient visit to that hospital, he was suspected to have Parkinsonism and started anti-parkinsonian medications. The treatments were not effective, and he came to our outpatient clinic. The patient had a brother and sister who were normal. Neurological examination revealed that the patient had bradykinesia, masked face, stooped posture, Parkinsonian gait, and postural instability. The patient had no pyramidal signs or dystonia. The cognitive function test showed mild cognitive decline. Bilateral obliteration of dopamine transporter uptake was found on $^{18}$F-FP-CIT PET scan. EEG and laboratory tests including screening for Wilson disease were normal. Next generation sequencing revealed p.Ser356ArgfsTer56 (c.1066_1069delTCTG) frameshift mutation and p.Arg27His (c.80G>A) missense variant of the FBXO7 gene.

Conclusions: Complete obliteration in $^{18}$F-FP-CIT PET can be considered as a possible imaging biomarker of FBXO7 mutation.
Aims: Synaptic vesicle 2A protein (SV2A) imaging with positron emission tomography (PET) has linked the loss of functional synapses to the cognitive decline observed in Alzheimer's and Parkinson's diseases. However, synaptic density changes in healthy ageing are less studied. Thus, we used SV2A PET to study synaptic density in healthy mice of different ages.

Methods: Three age groups (4-5 months: n=7, 12-14 months: n=11, 17-19 months: n=8) of C57BL/6J mice were PET scanned with SV2A tracer \(^{18}\text{F}\)SynVesT-1 for 60 min followed by CT. Images were subsequently aligned with an MRI-based brain atlas. Regions of interest in the brain and heart were extracted to obtain time-activity curves (TACs). Brain retention of \(^{18}\text{F}\)SynVesT-1 was calculated using one-tissue compartmental modelling (1TCM) based on image-derived input function and presented as the volume of distribution (V\(_T\)).

Results: Brain concentrations of \(^{18}\text{F}\)SynVesT-1 were decreased in the oldest group compared with the two younger groups (Figure 1e). Interestingly, the highest brain retention appeared in mice aged 12-14 months. All age groups displayed the same blood concentrations (Figure 1f). Thus, the difference in brain exposure was not due to differences in blood exposure.

Conclusions: Consistent with human PET data, our data suggest that the synaptic density in healthy ageing mice remains stable during the majority of the mouse life span and does not decrease until very old age. Even then, the decrease is moderate. This information is important as preclinical PET may be used to test novel drug compounds. Therefore, that data is also likely to be translatable to higher species.
DOPAMINE TRANSPORTER ACTIVITY AND AUTONOMIC FUNCTION IN PARKINSON’S DISEASE

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Aims: Autonomic dysfunction in Parkinson disease (PD) is common and suggested correlation with striatal dopamine deficit. This study aimed to investigate the association between dopamine transporter (DAT) activity and autonomic function in PD.

Methods: PD patients who underwent DAT PET using F-18-FP-CIT were evaluated autonomic function using composite autonomic severity score (CASS) and I-123 MIBG cardiac scan. A total 194 patients with mild PD (age 67.2±10.5, 112 women, Hoehn and Yahr stage (HY) 2.2±0.4) were recruited. DAT activity of striatum was compared based on delayed heart-to-mediastinum ratio (HMR) from I-123 MIBG cardiac scan and CASS.

Results: There was no difference in clinical characteristics between HMR groups. However, patients with abnormal HMR showed significantly increased CASS and lower DAT. Based on CASS, patients were grouped to mild (0-3), moderate (4-6), and severe (7-10) group. Mild CASS group was younger, and showed higher MMSE and lower HY than moderate and severe CASS groups. Mild CASS group also showed significantly increased HMR and DAT compared to those of moderate and severe CASS groups. Bivariate correlation analyses showed correlation of DAT with HMR and CASS, and there were also strong correlation of DAT with demographic and clinical characteristics. To confirm the correlation, multiple regression analyses adding age, disease duration and HY as covariate were done and revealed significant association of DAT with HMR and CASS.

Conclusions: This study found strong association between autonomic dysfunction and with striatal DAT activity in PD. Further studies are warranted to investigate the dopaminergic role in pathophysiology of autonomic dysfunction in PD.
POSTERS

APATHY IS ASSOCIATED WITH BAD NIGHT SLEEP IN DE-NOVO, UNTREATED PARKINSON’S DISEASE

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Aims: Sleep disturbance is one of the most common non-motor symptoms in Parkinson’s disease (PD) and seriously impacts the quality of life in patients with PD and their caregivers. We aim to investigate the clinical features, in particular mood symptoms, associated with night sleep disturbance in patients with de-novo, untreated PD.

Methods: A total of 108 patients with de-novo, untreated PD were included in this study. The night sleep disturbance was evaluated by night sleep subscale of SCOPA-Sleep. The ADL and parkinsonism were evaluated by UPDRS II and III. The cognition was evaluated by MMSE, in addition to FAB. The depression, anxiety, and apathy were assessed by GDS, BAI, and AES, respectively. Early perfusion and dopamine transporter imaging of F-18 FP-CIT PET/CT were obtained and statistical parametric mapping analysis were performed.

Results: Night sleep sub-score of SCOPA-Sleep was correlated with AES score (P = 0.014), BAI score (P = 0.014), and GDS score (P = 0.023). The twenty-six patients (24.1%) were classified as bad night sleep. The patients with bad night sleep had more apathy than those with good night sleep (P = 0.013). Binary logistic regression analysis showed that higher AES score was associated with bad night sleep (P = 0.016). And there showed increased perfusion in left posterior cingulate in PD with sleep disturbance and apathy compared with the PD with sleep disturbance only.

Conclusions: This study identified that night sleep disturbance is related to mood disorders, in particular apathy, in patients with de-novo, untreated PD.
DOPAMINERGIC NETWORKS RECONFIGURATIONS IN PRODROMAL DEMENTIA WITH LEWY BODIES

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Aims: dopaminergic nigrostriatal and extranigrostriatal deficits are a core feature of dementia with Lewy bodies (DLB) but are still debated in the recently defined prodromal phases of the disease. Objective of the study was to examine local and long-distance dopaminergic alterations in prodromal DLB patients and compare them with patients with DLB dementia and Parkinson’s disease (PD).

Methods: twenty patients with mild cognitive impairment due to Lewy bodies (MCI-LB), 29 DLB patients, 79 PD patients without cognitive deficits, and 73 healthy controls (CG) entered the study. Each patients underwent a standardized neurological examination and [123I]FP-CIT-SPECT imaging. The occipital-adjusted specific to nondisplaceable binding ratio (SBR) in cortical and subcortical regions were compared between groups adjusting for the effects of age, sex, disease duration, and motor impairment. Molecular connectivity analyses within the ventral and dorsal dopaminergic networks were evaluated by partial-correlation analysis.

Results: MCI-LB were characterized by similar putamen and caudate deficits com Thalamic SBR appeared to be selectively reduced in DLB and MCI-LB compared to CG and PD. Molecular connectivity assessment revealed a widespread loss of inter-connections among subcortical and cortical targets of DA networks in the three clinical groups. The MCI-LB group showed strong and widespread metabolic connectivity reconfigurations in the two networks with significant loss and connections compared to the CG.

Conclusions: MCI-LB cohort was characterized by early local and system-level alterations of the dopaminergic networks. MCI-LB and DLB shared specific alterations compared to PD. The prominent molecular connectivity changes in MCI-LB might suggest neural compensation mechanisms in the prodromal phase.
POSTERS

CSF ALPHA-SYNUCLEIN PMCA ASSAY: SENSITIVITY IN AUTOPSY CONFIRMED CASES DEPENDS ON DISTRIBUTION OF LEWY BODY PATHOLOGY


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Aims: To examine the sensitivity and specificity of alpha-synuclein protein misfolding cyclic amplification (αSyn-PMCA) in detecting alpha-synuclein seeding species from antemortem and postmortem CSF using a multicenter autopsy-validated cohort with a spectrum of Lewy body pathology (LBP).

Methods: 120 subjects with CSF and neuropathological assessments from University of California San Diego and Oregon Health & Science University were analyzed using αSyn-PMCA: 55 were LBP negative (LBP-), 7 had limbic-stage LBP, 38 had neocortical-stage LBP, and 20 had amygdala-predominant LBP. 56/120 had postmortem CSF collected (27 LBP-, 29 LBP+: 1 limbic, 19 neocortical, 9 amygdala-predominant).

Results: Average time from antemortem CSF sampling to death was 4.9 years (SD 3.4 years). Using antemortem CSF samples, 0/55 LBP- cases had positive αSyn-PMCA results, yielding assay specificity of 100% (95%CI [94-100]). αSyn-PMCA had overall sensitivity of 89% (95%CI [76-96]) for detecting limbic or neocortical stage LBP (40/45), but only 10% (95%CI [2-30]) for detecting amygdala-predominant LBP (2/20). Among postmortem CSF samples, 3/27 LBP- had a positive αSyn-PMCA result, yielding specificity of 89% (95%CI [71-98]). Sensitivity was 75% (95%CI [51-91]) for detecting limbic or neocortical stage LBP (15/20) and 22% sensitivity (95%CI [4-55]) for detecting amygdala predominant LBP (2/9).

Conclusions: In this multicenter autopsy-validated study of αSyn-PMCA using antemortem and postmortem CSF across a spectrum of LBP, we observe a markedly decreased sensitivity of detecting amygdala-predominant LBP. Possible explanations include lower concentrations of alpha-synuclein seeding species or different alpha-synuclein conformations with variable seeding activity in these cases. Further studies of alpha-synuclein conformers in amygdala-predominant LBP are warranted.
GLYMPHATIC FUNCTION IN PARKINSON’S DISEASE

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Aims: The glymphatic system (GS) is involved in the clearance of solutes and peptides from the brain through the exchange of cerebrospinal fluid (CSF) and interstitial fluid, mediated by the water channel aquaporin 4 (AQP4). Interestingly, GS is impaired in Alzheimer’s disease, and is involved in the clearance of amyloid beta from the brain. However, how the GS is affected in PD and its role in the removal from the brain of α-synuclein (α-syn) has not been established yet. Here we investigated whether GS is affected in PD, and whether it plays a role in PD pathogenesis.

Methods: We injected fluorescent tracers in the CSF at the cisterna magna (CM) to track its distribution in the brain and periphery, in two mouse models of PD: the 6-hydroxydopamine (6-OHDA) lesion and the human-α-synuclein/pre-formed fibrils (h-α-syn-PFF) which mimic the late and pre-symptomatic stages of PD, respectively.

Results: Our results suggested a trend towards decreased CSF influx in the brain of 6-OHDA mice, suggesting that neurodegeneration has a negative impact on GS. Similarly, we found a trend towards decreased CSF tracer influx in the brain and efflux to peripheral tissues in the h-α-syn-PFF model of PD. Furthermore, the clearance of α-syn injected in the brain followed glymphatic pathways.

Conclusions: Taken together, these results support the hypothesis that an impairment of glymphatic function, e.g. induced by physiological aging, might contribute to α-syn accumulation in the brain in pre-symptomatic stages of PD.
Aims: GM1 ganglioside is involved in regulation of Glial Derived Neurotrophic Factor signaling. Earlier research on GM1 in Parkinson’s Disease (PD) patients have emphasized on its deficiency that predisposes people to PD. The decrease in GM1 level causes degeneration of dopaminergic neurons. In this study, GM1 in peripheral blood mononuclear cells (PBMCs) and colon of PD patients was analyzed.

Methods: A total of 12 PBMC and 18 colon samples were assayed. Lipid content of PBMCs and colon was extracted using chloroform: methanol and applied to the high-performance thin-layer chromatography (HPTLC) plates. Following HPTLC development and Cholera toxin B(CtxB)- horseradish peroxidase (HRP) application, GM1 and the other gangliosides were revealed by enhanced chemiluminescence (ECL) reagent and quantified by densitometry.

Results: The HPTLC analysis indicated that GM1 along with GD1a are deficient in PD patients’ sample both PBMC and colon tissue, when compared to age-matched control sample. Although PBMCs are not directly involved in neuronal degeneration or its functioning, it is interesting as DA, tyrosine hydroxylase, and the DA transporter decrease in PBMCs of PD patients and now with the help of current study we speculate that GM1 also decreases in PD.

Conclusions: GM1 concentration declines with age, however, the decline is prominent in PD. The present study shows GM1 deficiency in non-CNS PD tissues, PBMCs and colon, in correlation to the hypothesis that GM1 manifests systemic deficiency in all tissues of the PD patient’s body. Hence, this constitutes a promising method for early diagnosis of PD and GM1 replacement offers promise as a disease-altering therapy.
Aims: In this study we aimed to characterize the tear fluid miRNAome of patients with Parkinson’s disease (PD) and atypical Parkinson syndromes (aPS) using quantitative real-time polymerase chain reaction (qRT-PCR). Based on the differential abundance of miRNAs, we aimed to develop a miRNA signature capable of differentiating these disease entities.

Methods: Tear fluid samples from 19 patients with PD, 7 patients with multisystem atrophy (MSA) and 10 patients with progressive supranuclear palsy (PSP) as well as 10 healthy age-matched control subjects were obtained at one center. RNA was extracted, pooled by disease entity and subjected to miRNA-quantification using the hsa-miRNome MicroRNA Profiling Kit (System Biosciences) detecting 1113 miRNAs. Differentially expressed miRNAs were then validated in individual patient samples using qRT-PCR.

Results: Of all 1113 quantified miRNAs, 262 were amplified in all conditions. Our analyses revealed 25 miRNAs that were not detected in controls when compared to either PD or aPS. 6 miRNAs were unique to PD (miR-597-5p, miR-670-5p, miR-4290, miR-516-5p, miR-523-5p and miR-let-7d-3p). MiR-323-3p was only found in MSA, whereas miR-206 and miR-490-5p were found in all conditions but PSP.

Conclusions: Tear fluid is an easily collectable biofluid and miRNA can be successfully extracted. Our results suggest that miRNA from tear fluid could be used to differentiate PD and aPS through expression patterns. Further studies in larger cohorts are required to validate our results and assess the value of tear fluid miRNA as biomarker for PD and aPS.
Aims: Using a multi-cohort, Discovery-Replication-Validation design, we sought to identify new plasma biomarkers that predict which PD individuals will experience cognitive decline.

Methods: In 108 Discovery Cohort PD individuals and 83 Replication Cohort PD individuals, we measured 940 plasma proteins on an aptamer-based platform. Using proteins associating with subsequent cognitive decline in both cohorts, we trained a logistic regression-based model to predict which PD patients showed fast (>=1 point drop/year on Montreal Cognitive Assessment (MoCA)) vs. slow (<1 point drop/year on MoCA) cognitive decline in the Discovery Cohort and tested it in the Replication Cohort. We developed alternate assays for the top 3 proteins and confirmed their ability to predict cognitive decline – as defined by change in MoCA or development of incident mild cognitive impairment (MCI) or dementia – in a 118-PD patient Validation Cohort. We used Mendelian randomization to investigate the top plasma biomarker for causal influence.

Results: A model using only 3 proteins (MIA, CRP, albumin) separated Fast vs. Slow cognitive decline subgroups with an AUC of 0.75 in the Replication Cohort and 0.80 in the Validation Cohort. Validation Cohort PD individuals in the top quartile of risk for cognitive decline predicted by the model were four times more likely to develop incident MCI or dementia than those in the lowest quartile. Genotypes at rs2233154 associated with MIA levels and cognitive decline, providing evidence for MIA’s causal influence.

Conclusions: An easily-obtained plasma-based predictor identifies PD individuals at risk for cognitive decline. MIA may participate in causal pathways related to development of cognitive impairment.
BRAIN-DERIVED EXTRACELLULAR VESICLE-ASSOCIATED ALPHA-SYNUCLEIN AS A POTENTIAL BLOOD BIOMARKER FOR SYNUCLEINOPATHIES

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Aims: Alpha-synuclein (aSyn) pathology is a hallmark of synucleinopathies including Parkinson’s disease and dementia with Lewy-bodies (DLB). Although easily detectable in the blood, aSyn originates from diverse cell types, which limits the measurement’s specificity for protein derived from the brain. We test whether aSyn associated with brain-derived extracellular vesicles (BDEs) in plasma can serve as a biomarker for synucleinopathies.

Methods: We quantified aSyn associated with BDEs using two strategies. First intact BDEs were captured on beads and detected using antibodies against total or phosphorylated aSyn (S129P), to exclusively measure aSyn on NDE membranes. Second, NDEs were isolated using NeuroDex proprietary procedure (ExoSORT™), and total aSyn was measured by MesoScale, and pS129- aSyn was measured by ELISA.

Results: The membrane-bound aSyn measurements generated a linear, dilution-dependent signal, which was antibody-specific and abolished by detergent. In a cohort of 25 PD and 17 control samples, PD had a significant increase in aSyn association with oligodendrocyte and microglia EVs (P=0.001 and 0.02 respectively), but not erythrocyte and neuronal EVs (P=0.26 and 0.87 respectively) in PD samples. MSD measurements of aSyn in isolated BDEs detected much lower concentration compared to plasma, and significant separation between PD and controls. Larger cross-sectional and longitudinal samples cohort of PD, DLB, and multiple system atrophy is being tested.

Conclusions: aSyn and pS129- aSyn assessments in blood BDEs provide a novel approach to improve cell-type specificity and shows promise as a specific biomarker for synucleinopathies. If validated, it can provide a valuable tool to improve the design and execution of clinical trials.
Aims: Alpha-synuclein seed amplification assay (αSyn-SAA, also known as RT-QuIC or PMCA) is a promising diagnostic tool for Parkinson’s disease (PD), enabling premortem detection of seeding-competent α-synuclein aggregates. We evaluated αSyn-SAA performance and reproducibility through independent analysis of a cerebrospinal fluid (CSF) panel by three separate laboratories.

Methods: CSF samples from a balanced subset of Parkinson’s Progression Markers Initiative (PPMI) participants (30 de novo PD, 30 healthy control [HC], and 20 SWEDD) from baseline and year 3 visits (for PD and HC groups) were blindly analyzed according to each laboratories’ established, optimized αSyn-SAA protocols. Secondary analyses of αSyn-SAA kinetic parameters and end-point dilutions were also performed.

Results: All three αSyn-SAA protocols achieved high diagnostic performance (sensitivity ranging from 86% to 96%, and specificity from 93% to 100%). They were also concordant for samples from PD individuals whose clinical diagnosis was later altered to non-PD through clinical consensus. All three assays also showed αSyn-SAA-positivity at baseline for 2 SWEDD subjects whose DaTScan changed from normal at baseline to abnormal later, suggesting the potential to diagnose prodromal PD. The αSyn-SAA kinetic parameters and end-point dilutions did not reveal consistent correlations to clinical features.

Conclusions: Results confirm the diagnostic accuracy and reproducibility of αSyn-SAA to be comparable or superior to existing methods. Assay kinetics or end-point dilutions, however, did not inform disease subtype, severity, or progression in this small sample set. Results of this study are being extended through analyses of a much larger PPMI sample set, including PD, HC, prodromal and genetic cohorts.
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POSTERS

DETECTION OF BLOOD EXOSOME ACETYLCHOLINESTERASE ACTIVITY IN PARKINSON'S DISEASE PATIENTS

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Aims: Degeneration of dopaminergic neurons has been recognized as one of the major pathophysiological features of Parkinson’s Disease (PD). Several studies have shown that cholinergic denervation also could be occurred in PD patients. In particular, molecular neuroimaging demonstrated the decline of acetylcholinesterase (AChE) activity in the central and peripheral nervous systems. However, there has been no attempt to measure AChE activity in exosomes, small extracellular vesicles produced in various cell types.

Methods: Herein, we isolated exosomes from the plasma of 34 PD patients and 29 healthy control (HC) by ultracentrifugation. Exosomal AChE activity and α-synuclein (α-syn) were quantified and analyzed the relationship with clinical parameters.

Results: Interestingly, AChE activity in the exosome was statistically reduced in PD patients compared to normal controls (P = 0.002). Also, exosomal AChE activity showed a strong negative correlation with clinical severity, including UPDRS and H&Y scores. On the other hand, exosomal α-syn revealed no difference between the two groups.

Conclusions: Our results support the occurrence of cholinergic dysfunction in PD and implicate its relationship with disease progression, especially motor deficit. In addition, the measurement of exosomal AChE activity with further advanced exosome isolation techniques could be used as a reliable biomarker for the early diagnosis and prognosis of PD.
ALPHA-SYNUCLEIN IN EXTRACELLULAR VESICLE FRACTION FROM PLASMA IS NOT A PROMISING BIOMARKER FOR PARKINSON’S DISEASE

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Aims: There is a high need for biomarkers to confirm and follow α-Synuclein pathology in Parkinson’s disease (PD) patients. Recent studies detected α-Synuclein in EV fractions purified from human blood and showed increased levels in PD. However, a thorough characterization of EV α-Synuclein and independent validation of increased levels in PD are missing.

Methods: We developed and validated a workflow for total EV purification from human plasma using size-exclusion chromatography (SEC) and analyzed the location of α-Synuclein by ultrafiltration and ultracentrifugation. Finally, this method was applied to plasma from 25 healthy controls (HC) and 25 idiopathic PD (iPD) patients.

Results: A small fraction of α-Synuclein in human plasma co-migrated with EV markers during SEC while the majority of α-Synuclein was separated from the EV fraction. Ultracentrifugation as well as ultrafiltration of SEC-purified plasma EVs separated α-Synuclein from EV markers suggesting no or transient interaction between α-Synuclein and plasma EVs. Levels of α-Synuclein in the plasma EV fraction showed no significant difference between iPD and HC. α-Synuclein in the EV fraction was significantly correlated with the EV markers CD-9 and Flotillin-1 and with α-Synuclein in raw plasma.

Conclusions: Our results suggest that the majority of α-Synuclein in SEC-purified plasma EVs is located outside of EVs likely interacting in a transient fashion. No change of α-Synuclein levels in the EV fraction of PD vs. HC and high correlation with EV markers do not support utility as biomarker for PD.
SEED AMPLIFICATION ASSAY (SAA) METHOD AS A DIAGNOSTIC TOOL FOR PARKINSONIAN DISORDERS

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Aims: To assess the ability of the alpha-synuclein seed amplification assay (aSyn-SAA) method to detect alpha-synuclein (aSyn) aggregates in cerebrospinal fluid (CSF) samples and to discriminate between different movement disorders.

Methods: This study was performed with CSF samples from two independent cohorts comprised of healthy controls (n=45), and patients with Parkinson’s disease (PD) (n=56), corticobasal degeneration (CBD) (n=14), progressive supranuclear palsy (PSP) (n=30), or multiple system atrophy (MSA) (n=34). Presence of aSyn aggregates was assessed by the aSyn-SAA method, which can amplify small amounts of aggregates to levels detectable by thioflavin-T.

Results: The assay successfully detected aSyn aggregates in CSF samples. More specifically, 96% of PD samples were positive while tauopathy movement disorders, PSP and CBD, were mostly negative. MSA samples, even when discriminating between different disease subgroups, showed mixed results, which is consistent with previous studies. This might be due to the heterogeneity of the disease or inaccurate clinical diagnosis. It was also determined that most of the healthy control samples were negative. It is not clear whether the small number positive cases represent false positive results, incidental Lewy body pathology in aged individuals or early disease stages.

Conclusions: These findings suggest that the aSyn-SAA method is a sensitive assay for the detection of aSyn seeds in patients with PD. In addition, we determined that it can be a useful tool in discriminating PD from other movement disorders such as CBD and PSP.
DEVELOPMENT OF SENSITIVE MASS-SPECTROMETRIC ASSAYS FOR CANDIDATE BIOMARKERS IN DEMENTIA WITH LEWY BODIES

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Aims: Based on findings from the MIRIADE consortium, biomarker candidates for dementia with Lewy bodies (DLB) were selected according to their ability to differentiate between DLB, Alzheimer’s disease, and frontotemporal dementia (unpublished data). These included dopa decarboxylase (DDC), corticotropin-releasing hormone (CRH), and beta-glucocerebrosidase (GBA) proteins. The aim of this project was to develop a sensitive targeted mass-spectrometric assay to quantify novel and disease-specific biomarkers for DLB in cerebrospinal fluid (CSF).

Methods: Proteotypic peptides were selected and liquid chromatography (LC) coupled to parallel reaction monitoring (PRM) mass spectrometric methods were developed for the proteins.

Results: Isotope-labelled peptides from DDC, CRH and GBA were spiked in a healthy control (HC) CSF pool. The low abundance of the targets required the use of nanoflow-LC coupled to a Quadrupole-Orbitrap hybrid instrument for their detection by PRM in 5 μL CSF. This approach was successful in detecting and quantifying CRH and GBA (both present at only a few tens of attomoles per μL of HC CSF), but not DDC. Thus, a HC CSF pool (2 mL) was used to isolate DDC by immunoprecipitation and samples were analyzed by PRM with nanoflow-LC, which detected DDC peptides. Reproducibility for the PRM methods for CRH and GBA was assessed, and a pilot study was performed.

Conclusions: We have developed a sensitive PRM method to measure CRH and GBA in 5 μL CSF. Furthermore, we have developed a PRM method for DDC based on immunoprecipitation in 2 mL CSF.
RAPID AND SPECIFIC QUANTITATION OF CSF SEEDED ALPHA-SYNUCLEIN CORRELATED WITH DISEASE SEVERITY IN PARKINSON’S DISEASE PATIENTS

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Aims: Definitive diagnostic criteria of synucleinopathies mainly rely on postmortem finding of disease-associated pathology in the affected brain regions, mainly containing alpha-synuclein (alpha-Syn) as misfolded protein aggregates. Here we present an improved method of detecting alpha-Syn disease-associated seeds/oligomers by combining real-time quaking-induced conversion (RT-QuIC) with enzyme-linked immunosorbent assay (ELISA) and analyzing brain and CSF samples from PD patients and controls.

Methods: We initially analyzed two sets of brain homogenates from synucleinopathies and non-synucleinopathies, were analyzed using a combination of both RT-QuIC to analyze the samples and oligomeric-specific ELISA to quantify seeded alpha-Syn oligomers at multiple timepoints (20 h and 60 h). We then analyzed CSF samples from 62 PD patients and 34 healthy control subjects. The correlation between seeded alpha-Syn oligomers and disease severity was also explored.

Results: Oligomeric-ELISA demonstrated high sensitivity and specificity quantifying RT-QuIC end product seeded by brain homogenates from patients with PD and DLB. Seeding activity of PD and DLB brain homogenates was detected earlier using when combining RT-QuIC and oligomeric-ELISA. Moreover, CSF seeded αSyn levels correlated with RT-QuIC data and robustly discriminated PD patients from controls. More interestingly, CSF seeded αSyn oligomers correlated with the severity of the clinical symptoms of PD measured by UPDRS-motor (r = 0.58, P<0.001) and H&Y scores (r = 0.43, P<0.01).

Conclusions: The development of high-throughput techniques is highly needed for the identification of potential PD biomarkers. Our study presents an improved approach for specific and sensitive quantitation of seeded alpha-Syn oligomers as a potential biomarker for PD.
THE INFLUENCE OF ANTIDEPRESSANTS ON NON-REM SLEEP HYPERTONIA, A BIOMARKER FOR PARKINSONIAN-SPECTRUM DISORDERS

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**Aims:** The severity of REM sleep without atonia, a prodromal biomarker for synucleinopathy-related neurodegenerative disorders, is influenced by selective serotonin reuptake inhibitor (SSRI) use. This study investigates whether SSRIs similarly impacts the severity of Non-REM sleep with hypertonia (NRH), a biomarker independently associated with Parkinson-spectrum disorders (PSD).

**Methods:** The relationship between NRH and SSRIs was evaluated in PSD patients with Lewy Bodies/Parkinson’s Disease Dementia (n=15), Parkinson’s Disease (n=14), isolated REM sleep behavior disorder (n=19), or progressive supranuclear palsy (n=12), and non-PSD subjects with Alzheimer’s Disease (n=22), mild cognitive impairment (n=35), or normal cognition (n=61). In-home studies were conducted with the Sleep Profiler in all participants except iRBD patients, who underwent SP recordings during in-laboratory polysomnography. Statistical analyses included multiple logistic regression, Chi-squared, and Mann-Whitney U-tests using an auto-scored abnormal-NRH threshold of >5% of sleep time.

**Results:** In the 178 records, abnormal-NRH without SSRI use (NRH-only) was observed in 67 records while abnormal-NRH with SSRI use (NRH-SSRI) occurred in 20 cases. The proportion of SSRI use trended higher in the PSD patients (28% vs. 17%, P=0.08). Abnormal-NRH was associated with the PSD group (P<0.0001), independent of sex (P=0.08), age (P=0.61) and SSRI use (P=0.09). The proportion of cases with abnormal-NRH taking SSRIs were similar in the PSD and non-PSD groups (35 vs. 19%, P=0.26). No differences in NRH severity were observed in the NRH-only vs. NRH-SSRI groups (15+8.7 vs 18+10.3%, P=0.238).

**Conclusions:** SSRI use trended higher in PSD patients but does not appear to significantly influence abnormal-NRH prevalence or severity.
EMOTIONAL PROCESSING AND FACE-SPECIFIC EVENT-RELATED POTENTIALS IN PARKINSON DISEASE

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Aims: Parkinson's disease (PD) is associated with impaired emotional information processing. But it is still not clear how this impairment is related to the severity and phenotype of the disease.

Methods: In this study, we used the images of neutral and happy faces (NimStim Set of Facial Expressions) to elicit the N170 component of visual event-related potentials (ERP). 150 patients with PD (Hoehn-Yahr 2.0-3.0) and 20 control participants without neurological disorders participated in the experiment. To identify non-motor subtypes of PD patients we used the cluster analysis based on demographic and clinical data (Unified Parkinson's Disease Rating Scale, Non-Motor Symptoms Scale).

Results: 4 clusters were extracted (numbers 1 to 4 corresponds to an increase in UPDRS scale). Prominent non-motor symptoms were typical for clusters 2 and 4 only. There was no difference in N170 latency between all clusters and the control group. The amplitude of this component was significantly lower in controls compared to clusters 2 and 4 in right temporal and occipital sites for happy faces. An increase in N170 amplitude was additionally registered in response to neutral stimuli in patients of cluster 4 (the most severe PD), and this difference was bilateral.

Conclusions: Thus, our results show that the face processing subsystem is mostly impaired in patients with higher severity of non-motor symptoms, and N170 amplitude in response to face images may be useful for PD non-motor subtypes classification.
Aims: Decreased sleep spindle oscillations were previously associated with cognitive decline in older adults, increased tau levels, and phenoconversion to dementia in patients with Parkinson's disease (PD). We aim to analyze whether quantitative sleep spindle measures are associated with particular neurodegenerative disorders/syndromes.

Methods: Spindle-durations (i.e., sum of spindle lengths; minutes) were ascertained in patients broadly characterized as presumed Parkinsonian-Spectrum disorders (PSD), which included the subgroups dementia with Lewy Bodies/Parkinson Disease Dementia (DLB/PDD, n=15), PD (n=14), isolated REM sleep behavior disorder (iRBD, n=19), progressive supranuclear palsy (PSP, n=12), and compared with non-PSD subgroups Alzheimer’s Disease dementia (AD, n=22), mild cognitive impairment (MCI, n=35), and normal cognition (NC, n=61). In-home Sleep Profiler studies were conducted in all participants except iRBD patients, who had Sleep Profiler recorded during in-lab polysomnography. The automated spindle detection algorithms recognized temporal excursions in the alpha (8-12 Hz) and sigma (12-16 Hz) power of 250 milliseconds or greater. Statistical analyses included multiple logistic regression and Mann-Whitney U-tests.

Results: Lower spindle-duration was independently associated with PSD (P=0.019, OR 1.08, 95%-CI 1.01-1.14) vs. the non-PSD group, but unassociated with age (P=0.09, OR 1.03, 95%-CI 0.99-1.07). Spindle-durations were reduced in PSP (0.9+2.1) and DLB/PDD (2.0+5.1) subgroups when individually compared to AD (3.2+7.1), iRBD (3.3+3.4), PD (5.3+6.6), MCI (5.3+9.7), and NC (8.0+11.1)(all P<0.05). AD patients also exhibited lower spindle-durations than NC (P=0.03).

Conclusions: Decreased sleep spindle-duration was independently associated with PSP and DLB/PDD, and in AD. Reduced sleep spindle duration may be a distinct sleep biomarker for those disorders associated with prominent, thalamocortical dysfunction.
EVENT-RELATED OSCILLATIONS IN PATIENTS WITH DEMENTIA WITH LEWY BODIES WITH AND WITHOUT MUTATIONS IN THE GBA GENE

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\textbf{Aims:} To explore the differences in the structure of event-related oscillations (ERO) elicited by a visual oddball task in Dementia with Lewy bodies (DLB) patients with and without mutations in the GBA gene.

\textbf{Methods:} EEG was recorded during a visual oddball task in 19 Ashkenazi Jewish patients with DLB who underwent genotyping for mutations in the GBA gene. Time-frequency power and inter-trial phase clustering were calculated from the results of Morlet wavelet convolution.

\textbf{Results:} Eight patients were carriers of the N370S mutation in the GBA gene and 11 were non-carriers. Carriers were younger (67.0±4.5yrs vs. 75.1±5.2, p=0.004), had similar disease duration (4.4±3.1yrs vs. 3.6±1.8yrs), cognitive function (MoCA score: 17.8±6.2 vs. 21.0±5.0) and motor symptoms (MDS-UPDRS-III: 33.4±17.5 vs. 36.2±16.0). Task performance was comparable between groups, including similar reaction time and reaction time variability. Within-group EEG analysis revealed that in non-carriers, both event-related power and phase coherence were increased in the delta band as compared to the baseline period (p<0.009, Cohen's d>1.0). In GBA mutation carriers only event-related phase coherence, but not power significantly increased compared to the baseline period. Between-group analysis revealed that event-related power was decreased in carriers compared to non-carriers in delta band at Fz and Cz (p<0.04, Cohen's d<-0.9).

\textbf{Conclusions:} Our findings show reduced event-related power in DLB patients with the N370S mutation in the GBA gene compared to non-carriers, potentially signifying more pronounced network dysfunction. Interestingly, behavioral performance was comparable, and event-related coherence was intact in both groups, suggesting a possible compensatory effect that may involve other brain regions.
Aims: This study aimed to add to previous knowledge by describing the natural course of parkinsonism in autopsy-verified patients. More information is needed for future refinement of the parkinsonism core feature of DLB.

Methods: The Dementia Study of Western Norway included patients with mild dementia who were followed annually from diagnosis until death. Patients with a neuropathologically verified diagnosis of Alzheimer’s disease (AD) (n=31) or DLB (n=16) were included in this substudy. Parkinsonism was assessed with the Unified Parkinson’s Disease Rating Scale (UPDRS) motor subscale at inclusion and repeated at each assessment. 15 DLB and 27 AD patients had at least two available scores. The Mann-Whitney and the Chi-square test were used.

Results: UPDRS motor scores were higher in DLB than AD at diagnosis (p=0.001) and the first (p<0.001), but not second follow-up (p=0.081). More AD than DLB patients had consistently low UPDRS motor scores: 19 (77%) and 16 (60%) of AD patients and 5 (33%) and 4 (27%) of DLB patients never reached a score of 10 and 5 respectively. The number of missing UPDRS scores increased over time. Individuals with missing UPDRS scores at follow-up had more severe dementia (higher Clinical Dementia Rating scale global scores).

Conclusions: DLB-patients with mild dementia have significantly more parkinsonism than their AD counterparts, but a significant proportion of DLB patients did not develop parkinsonism the first years after diagnosis. The UPDRS motor subscale is probably not an ideal instrument in moderate- to severe dementia.
NEUROANATOMIC SUBSTRATES OF ATTENTION PROCESSING SPEED IN EARLY DEMENTIA WITH LEWY BODIES (DLB): A WHITE-MATTER VOXEL-BASED MORPHOMETRY AND DIFFUSION TENSOR IMAGING STUDY

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Aims: The purpose of this study was to examine, in early DLB patients, the relationship between impaired attentional speed performances and white matter changes.

Methods: We administered the Trail Making Test A (TMTA) to 73 prodromal to moderate DLB patients (mean MMSE = 26.4) and 30 control subjects (mean MMSE = 28.9) to assess attention processing speed. Three-dimensional (3D) MRI and Diffusion-weighted images (DTI) were acquired for all participants and correlational analyses were performed in the patient group using WM-VBM, fractional anisotropy (FA) and mean diffusivity (MD). Based on JHU DTI-based white-matter atlas, correlations were also calculated between mean values of FA and MD coefficients, for relevant white matter tracts, and TMTA scores.

Results: Behavioral results showed significantly impaired performances in patients compared to control subjects (\(p = .004\)). Correlational analyses using WM-VBM revealed negative WM correlations in the anterior corpus callosum and in the anterior cingulum (\(p < .05\) FDR). Using DTI (\(p < .05\) FDR), correlated with TMTA scores, we found reduced FA in bilateral frontal and posterior regions, and increased MD in bilateral anterior and posterior networks. Correlations calculated for JHU DTI-based white matter tracts showed significant correlations with both FA and MD primarily in bilateral anterior corona radiata.

Conclusions: WM-VBM analysis revealed the involvement of the anterior corpus callosum and the cingulum. Changes in both FA and MD also correlate with impaired attentional speed. Our results also revealed a disruption of white matter tracts affecting primarily the anterior corona radiata.
ASSESSMENT OF THE PENTAGON COPYING TEST IN PARKINSON’S DISEASE WITH DIGITIZING TABLET

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Aims: We used a digitizing tablet during the pentagon copying test (PCT) as a tool for assessing early cognitive deficits in patients with Parkinson’s disease without dementia. We also aimed to uncover the neural correlates of the identified parameters.

Methods: We enrolled 27 PD patients without dementia and 25 age-matched healthy controls (HC). During parametrization of drawing, we focused on the commonly used features and compared both groups using the Mann-Whitney U test. Parameters with between-group differences were correlated with cognitive domains’ z-scores and were used as covariates in the whole-brain voxel-wise analysis using 3T MRI and voxel-based morphometry.

Results: The PD and HC groups differed in Shannon entropy, which quantifies the excessive in-air movements between two strokes (p = 0.003). In PD group, a correlation was found between the median of Shannon entropy and attention z-scores (R = -0.55, p = 0.006). The VBM showed an association between this parameter and gray matter volume in the right superior parietal lobe (SPL).

Conclusions: Using a digitizing tablet during pentagon drawing, we identified a novel entropy-based parameter that differed between HC and PD groups and correlated with the level of attention in PD patients. This in-air parameter was also linked with the gray matter volume variability of the right SPL.
AUTOMATICALLY DETERMINING DIADOCHOKINETIC RATE USING SONORITY LINE METHOD

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Aims: Verbal diadochokinesis (DDK) is a widely used clinical assessment of oral-motor function in movement disorders. We aim to contribute to automating this process using signal analysis and machine learning to derive alternating movement rate (AMR) and sequential movement rate (SMR).

Methods: 153 participants aged 18-65 completed automated DDK tests using the Neurovocalix platform. Participants repeated the syllables “pa”, “ta”, “ka” (SMR), or the sequence “pa ta ka” (AMR) twice for 10 seconds each at their maximum rate. 700 DDK utterances were manually scored. Signal analysis including discrete Fourier transformation (DFT), principal component analysis (PCA), and Gaussian filtering produced a sonority line for each utterance. Machine learning techniques including gradient-boosted decision trees, support vector machines (SVM), Gaussian naïve Bayes (GNB), and ensemble methods classified local maxima as syllables or outliers. 22,937 local maxima were manually labelled to produce the dataset for machine learning (18% of available data) and tested on the entire dataset. Postprocessing identified a continuous block of predicted syllable nuclei from which rate was calculated.

Results: Overall mean rate estimation accuracy was 93.5% (SD 7.5%), with SMR specifically having a mean estimation accuracy 95.8% (SD 5.2%). Predicted rate had an overall correlation of $r=.90$ with the ground truth, while SMR specifically had coefficient $r=.97$.

Conclusions: These results suggest that automatically estimating syllable rate can be done accurately using the sonority line method. This method works better on SMR than AMR. Future work may benefit from training on larger datasets, validation in patients and extended use of ensembles.
Aims: The aim of the present study is to evaluate the diagnostic accuracy of the LBCRS and the AT-DLB, as well as their capacity to enhance application and interpretation of the Consensus Criteria in a large cohort of patients referred to the Italian Centers for Cognitive Decline and Dementia.

Methods: In this study, LBCRS and AT-DLB were distributed to 135 Cognitive Decline and Dementia Centers (CDCD). We asked to administer the two questionnaires to all patients referred within the following three months, independently of the clinical diagnosis, and also to apply Consensus Criteria for DLB diagnosis, according to the results of each of the two toolkits, to all subjects.

Results: A total of 23 Centers participated to the survey, and 2006 patients were enrolled. Diagnosis of dementia was not confirmed in 152 (7.58%) subjects, who were hence excluded from the cohort. Of the 1854 remaining patients, 1048 (56.53%) were female; the mean age of the sample was 75.06±14.58 years. LBCRS toolkit showed a good reliability, with a Cronbach-alpha of 0.77. Furthermore, even removing variables from the construct, the value of the Cronbach-alpha was always ranging from 0.74 to 0.76. Conversely, AT-DLB toolkit Cronbach-alpha was 0.52 and, after the subtraction of the “cognitive fluctuation” criterion, was only 0.31.

Conclusions: In a clinical setting, the use of LBCRS questionnaire shows a good diagnostic accuracy for DLB diagnosis. Conversely, AT-DLB questionnaire seems to have lower performances, as compared. Further investigations should focus on the assessment of LBCRS capacity to identify DLB patients at prodromal stages.
VISUAL FUNCTIONS AND THEIR ASSOCIATION WITH VISUAL HALLUCINATIONS IN PATIENTS WITH DEMENTIA WITH LEWY BODIES

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Aims: It has been suggested that deficits in visual functions could partly underly visual hallucinations in dementia with Lewy bodies (DLB), but that hypothesis needs to be further investigated. We conducted a systematic review to summarise current state-of-the-art about the association between visual functions and visual hallucinations in DLB.

Methods: A systematic review was carried out following the PRISMA Statement. Pubmed, Scopus, PsycInfo and WOS were searched for primary articles published from 1996 to 2021. We included articles investigating the relationship between visual hallucinations and visual functions in probable or possible DLB patients. The risk of bias and quality of the included studies were assessed with the Critical Appraisal Skills Programme (CASP).

Results: A total of 310 studies were identified, of which 12 studies met our selection criteria. Patients with DLB and visual hallucinations performed worse in tasks of visual functioning compared to DLB without visual hallucinations. Patients with DLB who had severe deficits in visual functions more often had visual hallucinations as compared with those with mild visual deficits. Correlations were observed for visual hallucinations with deficits in visual functions as well as in attention.

Conclusions: The association between visual hallucinations and deficits in visual functions in DLB is complex and extends to other cognitive functions such as attention. These results could have implications for the diagnosis and clinical management of visual hallucinations in DLB. Futures studies that combine cognitive and neuroimaging data are needed to clarify the neural underpinnings of visual hallucinations and visual deficits in DLB.
POSTERS

DISEASE SPECIFIC GLOBAL CLINICAL RATING SCALES FOR LEWY-BODY DEMENTIA: LITERATURE REVIEW AND CONCEPT IDENTIFICATION

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Aims: The 2015 JPND report on harmonizing clinical and biomarker protocols for studies in Lewy-body dementia (LBD) recognized use of the Clinical Dementia Rating (CDR) and Clinical Global Impression, but noted neither is disease specific. It was suggested modifications should address other relevant domains of cognition, attention/wakefulness, psychiatric, motor, and functioning. Literature reviews to identify clinical outcome assessments (COAs) and measurement concepts are one early component of instrument development. We report data to inform iterative COA development.

Methods: Two data sources were used to explore use of COAs in clinical trials and clinical research in LBD. The first was clinicaltrials.gov (CT.gov). The second was a targeted literature review using terms related to COAs and Lewy body disease and dementia.

Results: The search of CT.gov identified 149 clinical trials in dementia with Lewy bodies or Parkinson’s disease dementia. Organized by measurement concepts, the most frequently used COAs were CDR (global; N=12), NPI (behavior/neuropsychological; N=27), MMSE (cognition; N=26). The literature review identified 20,695 results from peer reviewed journals of which the first 100 were selected sorted by relevance. Further filtering left 24 results for detailed evaluation. Again, the CDR (N=4), NPI (N=7), and MMSE (N=13) were prominent in this sample.

Conclusions: Detailed evaluation of measurement concepts within COAs can be used to identify items as a basis for iterative development of global scales such as the CDR and CGI, to create disease specific instruments. Supplementing these data with clinician, patient and caregiver input via qualitative research can then be used to finalize content for psychometric evaluation.
Aims: This study aimed to verify digital biomarkers for PD based on human dynamic characteristics (HDC) AI according to the PD severity levels.

Methods: A total of 90 participants, comprised of 30 mild-moderate PD, other 30 severe PD, and 30 age-matched healthy controls enrolled in the study. The Unified Parkinson's Disease Rating Scale (UPDRS) and Hoehn and Yahr scale (HY) were used to assess the disease severity. This study analyzed HDC parameters and PD digital biomarker (PD-DB score) of the participants by assessing the inertial measurement unit system. PD-DB score consists of R-score (Rigidity), T-score (Tremor), and B-score (Bradykinesia) and these were defined by big data based on HDC AI. We investigated the difference of HDC parameters and PD-DB score between participant groups and analyzed the correlation within PD severity score and PD-DB score.

Results: We found difference in HDC parameters and PD-DB score between the group in the stage of mild-moderate PD and the group in the stage of severe PD, as well as between the overall PD patients and the controls in HDC parameters and PD-DB score. Strong correlation was found between the UPDRS score and R-score, T-score and B-score respectively. The HDC AI discriminated PD patients from controls with over 80% accuracy and the mild-moderate PD from the severe PD with over 70% accuracy.

Conclusions: The HDC AI shows high potential as a simple and accurate diagnostic tool which can provide clinicians with support for a clinical decision for PD diagnosis and help build real-time big data of PD patients.
Aims: Numerous attempts to develop the early (presymptomatic) diagnosis of Parkinson’s disease (PD) remain unsuccessful. The aim of this study was to apply a novel approach – pharmacological challenge test. This test is based on a short-term reversible enhancement of the functional insufficiency of degrading nigrostriatal dopaminergic system to a threshold of motor symptoms appearance. An endogenous inhibitor of dopamine synthesis – monoiodotyrosine (MIT) could be used as provocative agent.

Methods: Mice model of presymptomatic PD was reproduced with subcutaneous injection of 18 mg/kg of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). One week later 100 mg/kg MIT was administered subcutaneously to MPTP-treated mice or to saline-treated control. Two hours later motor activity of mice was evaluated in an open-field test. Dopamine was assayed in the collected samples of striatum and substantia nigra by HPLC with electrochemical detection.

Results: Presymptomatic PD model was characterized by the absence of motor dysfunctions and subthreshold loss of striatal dopamine. Administration of MIT didn’t affect motor activity in control group, but in MPTP-treated mice reduced total distance in the open-field test by 55% compared to the mice that received neither MPTP nor MIT. This motor impairment was a result of a threshold decrease of striatal dopamine in MPTP+MIT group by 75%.

Conclusions: We obtained an experimental proof that MIT could be used as a provocative agent for the detection of latent nigrostriatal dysfunction in presymptomatic PD. The short-term and long-term safety of MIT-based challenge test should be evaluated in the future studies. This study was supported by the Russian Science Foundation (project No.20-75-00034).
Aims: Recent studies have shown accumulation and aggregation of alpha-synuclein in skin tissue of Parkinson's disease (PD) patients. To improve our understanding of alpha-synuclein pathology in the skin and to evaluate its potential as a future diagnostic biomarker, we assessed the frequency, localization and morphology of alpha-synuclein variants in postmortem skin tissue of clinically-diagnosed and pathologically-confirmed PD donors and non-neurological controls.

Methods: Sequential sections of post-mortem skin biopsies (n=8 PD, n=3 controls) from the scalp/C7 (back) region were stained with immunohistochemistry using a panel of antibodies targeted at total (Clone 42/alpha-synuclein), pSer129 (EP1536Y and P-syn/81A), aggregated (5G4) and C-terminal truncated-122 (A15127A) alpha-synuclein. Scoring was done by counting positive subdermal structures using QuPath.

Results: Most alpha-synuclein deposits were detected in PD skin tissue by staining for pSer129 alpha-synuclein (sensitivity: 100%) and were localized around sweat glands, blood vessels, arrector pili muscles and nerve fiber bundles. Other antibodies targeted at total (88%), C-terminal truncated (50%) and aggregated (38%) alpha-synuclein were less sensitive for detecting alpha-synuclein deposits in skin tissue from PD donors. Each antibody showed a unique profile in terms of localization and detected morphologies in the skin (thread-like neurites, bulgy neurites, dot-like and diffuse structures).

Conclusions: These results indicate that distinct localized staining patterns can be observed for each of the investigated antibodies, highlighting the importance of using an antibody panel to capture the diversity of alpha-synuclein morphology and pathology. The sensitivity of pSer129 alpha-synuclein detection in PD skin tissue highlights the potential of skin biopsies as a future diagnostic tool.
SENSITIVITY OF DETECTING ALPHA-SYNUCLEIN ACCUMULATION IN THE GASTROINTESTINAL TRACT AND TISSUE VOLUME EXAMINED

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Aims: The alpha-synuclein (AS) pathology of the gastrointestinal (GI) tract has the potential to be used as a biomarker, but low sensitivity makes it difficult to be used in practice. This study aimed to examine whether evaluation of larger tissue volume increases sensitivity of detecting AS pathology in the GI tract.

Methods: Nine patients with Parkinson's disease (PD) and idiopathic rapid eye movement sleep disorder (iRBD) who underwent GI operation and have both proximal and distal full-depth intestinal blocks archived in the pathology bank were included in the study. In addition, 5 subjects were selected from the control group. A total of 10 slides (5 serial sections from proximal and distal block respectively) per patient were analyzed.

Results: In the previous studies, pAS was positive in 5/9 patients (55.6%) and in 1/5 control (20%), which increased to in 8/9 patients (89%) but remained same in controls (20%) by this extensive examination. Positive findings were observed in both proximal and distal blocks in 66.7% of pAS(+) subjects, and in all 5 slides per block in 80.0% of pAS(+) blocks. Severity and distribution of positive findings were similar between patients with iRBD and PD.

Conclusions: Examining large tissue volume increases sensitivity of detecting AS accumulation in the GI tract. Lewy pathology in the stomach shows similar distribution and severity between patients with iRBD and PD. This study supports the fundamental limit of biopsied tissue and presence of the alternative pathway for synucleinopathy which does not follow the gut-to-brain progression.
CORTICOBASAL SYNDROME IN PATIENT WITH CLINICAL TAUPATHY-LIKE PHENOTYPE AND α-SYNUCLEIN AGGREGATES. EVIDENCE FROM THE TREDEM REGISTRY

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Aims: We present a patient with corticobasal syndrome (CBS) and Mild Cognitive Impairment (MCI) who showed the presence of α-synuclein aggregates in the CSF and in the olfactory mucosa samples. An 82-year-old male developed gestures of the left upper limb independent of the patient’s will, characteristics of alien limb phenomenon.

Methods: A clinical, neuropsychological, imaging and biomarkers evaluation, including tau and amyloid proteins levels in the CSF and RT-QuIC assay for α-synuclein both in the CSF and olfactory mucosa, along with a quantitative electroencephalography (QEEG) assessment were conducted.

Results: The patient, on the left side, presented with resting tremor, mild extrapiramidal hypertonus, mild bradykinesia and severe apraxia on the left upper limb. Brain MRI showed a knife-edge posterior parietal cortical atrophy prevalent on the right hemisphere. 18F-FDG PET imaging showed hypometabolism of the right lateral parietal, temporal cortex, precuneus and posterior cingulate cortex. The DaTscan showed a mild thinning of the posterior portion of the right putamen. Neuropsychological tests, performed annually for three consecutive years, showed memory and visual-perceptual deficits. CSF tau and amyloid measurements did not show clearly pathological values (Tau protein = 395 pg/mL; Aβ1–42/pTau181 ratio = 6.8) while RT-QuIC for α-synuclein in CSF and olfactory mucosa samples were positive. The QEEG analysis showed a dominant frequency varying between 6 and 10 Hzs in the posterior derivations, typical pattern of early stage Lewy body dementia (DLB).

Conclusions: Although in our patient the clinical diagnosis was of probable CBS, usually expression of tauopathies, unexpectedly RT-QuIC detected α-synuclein aggregates showing a probable α-synuclein pathology.
NOVEL KINETIC ASSAY SEEDING ABILITY RECOVERY (KASAR) PROTOCOL FOR UTILIZING FORMALIN-FIXED AND PARAFFIN-EMBEDDED SAMPLES IN ALPHA-SYNUCLEIN SEED AMPLIFICATION ASSAYS (SAA)

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Aims: Recent development of the alpha-synuclein seed amplification assays (SAA), including the ultrasensitive real time-quake inducing conversion (RT-QuIC) assay, has made it possible to detect misfolded alpha-synuclein in human tissues. Although formalin-fixed paraffin-embedded (FFPE) samples are plentiful in clinical settings, a tissue recovery protocol for FFPE samples is yet to be established. Previous experiments utilizing fixed or embedded tissues in SAA yielded inconsistent results. Thus, the objective of this study aims to develop an efficient methodology for utilizing FFPE samples in SAA biomarker discovery platform for various synucleinopathies including Parkinson’s disease (PD).

Methods: The KASAR protocol utilizes a recovery buffer and heat to mitigate the effects of formalin fixation on cellular components. The effects of KASAR treatment on RT-QuIC was examined using highly sensitive brain and submandibular gland (SMG) tissues from human autopsy specimens, as well as antemortem SMG needle biopsy samples.

Results: The KASAR protocol successfully recovered the seeding ability of formalin fixed and FFPE samples to nearly equal that of fresh tissue. Applying just a few nanograms of sample after treatment with the KASAR protocol has proven to be very effective at discriminating PD cases from healthy controls.

Conclusions: Improved detection of biomarker misfolded protein aggregates from formalin-fixed synucleinopathy case samples is a significant achievement. The KASAR protocol offers a process by which fixed or embedded tissues can reproducibly be analyzed using ultra-sensitive SAA, which opens vast libraries of preserved tissues and associated patient data for biomarker study. Study supported by MJFF and NINDS.
Aims: To evaluate if cognitive and motor features of patients with prodromal and overt dementia with Lewy bodies (DLB) are associated with the impairment of specific neurotransmitter circuits, evaluated in vivo with transcranial magnetic stimulation (TMS).

Methods: Fifty-one patients with DLB (twenty-five prodromal; twenty-six with dementia) underwent neuropsychological and clinical evaluation, with twenty-five patients having at least one follow-up evaluation. All patients underwent TMS assessment at baseline, with protocols assessing cholinergic circuits (short latency afferent inhibition – SAI), GABAergic circuits (short interval intracortical inhibition – SICI) and glutamatergic circuits (intracortical facilitation – ICF).

Results: Compared to HC, SICI, ICF and SAI resulted significantly impaired in both prodromal and overt DLB, with the latter showing a reduced SICI and SAI also compared to prodromal DLB. There was a significant correlation between motor deficits, evaluated with the UPDRS-III, and the impairment of GABAergic (SICI) \( r=0.729, p<0.001 \) and glutamatergic (ICF) \( r=0.608, p<0.001 \) circuits; global cognition, evaluated with the MMSE, correlated with the impairment of cholinergic (SAI) circuits \( r=-0.738, p<0.001 \). Worsening of cognitive functions at follow-up was associated with reduced cholinergic functions at baseline \( R^2=0.53\% , p<0.001 \).

Conclusions: These results suggest that motor and cognitive dysfunctions in prodromal and overt DLB depend on specific and independent neurotransmitter circuits.
Aims: Cognitive impairment is a debilitating symptom in Parkinson’s disease (PD), with high variability in onset, rate and trajectory of decline. We aimed to establish a powerful multivariate machine learning (ML) prediction model of cognitive function trajectory in Parkinson’s disease.

Methods: We subset PD cases based on MDS-criteria cognitive diagnosis to test outcomes of i) dementia conversion, or ii) any cognitive impairment over an eight year time span. Baseline data was organised into feature bins of clinical, biofluid (neurodegenerative csf markers) and genetic/epigenetic (genotypes, polygenic risk scores, DNA methylation). Subjects were split into 60% Training, 40% Testing and each feature class, as well as all features combined were tested using multiple machine learning models (Random Forest, Elasticnet, SVM-linear). Models were evaluated for prediction metrics (MCC, AUC) and individual model feature importance was assessed using shapley values.

Results: Clinical features alone showed best individual prediction (AUC = 0.85 – 0.92) although collated models with all feature types showed similar performance. Of both outcomes, overall cognitive impairment showed better sensitivity than dementia conversion prediction. Common features were observed across all models including known risk factors of cognitive tests (HVLT, MDS-UPDRS Part I, SDM) and age of onset but also measures of gastrointestinal and olfactory symptoms and DNA methylation at cg20813518.

Conclusions: ML Models showed good prediction of overall cognitive impairment and selected features associated with cognitive decline in PD and supported evidence of gastrointestinal and olfactory symptoms as risk factors. DNA methylation appeared to show potential as a contributory biomarker, when included with clinical features.
Aims: OBJECTIVES: Parkinson's disease (PD) is a progressive neurodegenerative disease affecting more than 10M people worldwide. However, current diagnosis relies on non-specific clinical signs that often lead to incorrect or late diagnosis. Therefore, recently, attention has been focused on the development of new methods and the search for new potential biomarkers that would allow us to detect the disease sooner. The finding that patients already in the pre-motor stage suffer from various dysfunctions of the gastrointestinal tract draws our attention to these organs.

Methods: METHODS: We focused on the early detection of pathology associated with the formation of aggregated proteins in gastrointestinal tissues. In a mouse model, PD-related pathology was induced by oral administration of Rotenone. Duodenal tissues were analyzed at 0,4,6,8,10,12 week intervals using Thioflavin S (ThS), a routinely used fluorescent probe to detect aggregated forms of proteins. In our analyzes, we combined fluorescence lifetime imaging (FLIM) and wholemount tissue analysis with a focus on mucosal and submucosal layers.

Results: RESULTS: In the duodenal tissues of rotenone-induced mice, we observed a lifetime of ThS fluorescence of 860ps (6 weeks), with a tendency to increase in a rotenone-treatment-dependent manner. We did not observe a similar effect in control animals. In addition, we used this methodology for the first time on wholemount tissue samples.

Conclusions: CONCLUSIONS: Our results indicate the suitability of using FLIM analysis even for complex samples as wholemount tissue, as well as the sensitivity of FLIM to detect gradual pathological changes associated with the formation of aggregated protein structures.
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**Aims:** Parkinson's disease (PD) and related disorders including Lewy body dementia (LBD) are characterized by the deposition of misfolded alpha-synuclein (αSyn) aggregates in affected brains. The overall objective of this study is to develop diagnostic biomarkers for PD and Lewy body dementias (LBD) using easily accessible peripheral tissues.

**Methods:** We have established a streamlined αSyn RT-QuIC assay platform allowing for a simplified analysis of αSyn aggregates in different types of biospecimens.

**Results:** Ultrasensitive and specific RT-QuIC detection of αSyn aggregates was achieved in million-fold diluted brain homogenates of PD and LBD cases. Our assay was further validated with CSF samples of 214 neuropathologically confirmed cases, yielding a sensitivity of 98% and a specificity of 100%. A single RT-QuIC assay protocol was employed uniformly to detect seeding activity of αSyn aggregates in PD samples across different tissue types including the brain, skin, and colon. We have recently performed αSyn RT-QuIC assay on skin biopsy and olfactory mucosa (OM) collected from patients affected by PD and other synucleinopathies. Our RT-QuIC analyses have shown that the disease-associated αSyn aggregates are readily detectable in the skin and OM from living patients. The seeding activity of the peripheral αSyn aggregates correlated with some clinical symptoms. Finally, we have revealed disease- and tissue-specific differences in the peripheral αSyn aggregates in PD and other synucleinopathies.

**Conclusions:** Our studies have demonstrated diagnostic applications of αSyn RT-QuIC using easily accessible peripheral tissues for early diagnosis of PD and LBD. Our research may facilitate the integration of the RT-QuIC biomarker assay into clinical practice.
Aims: Aggregated alpha-synuclein (aSyn), detected by seed amplification assays (SAA), is a promising candidate biomarker for Parkinson’s Disease (PD) diagnosis. Among various biospecimens tested thus far (brain, cerebrospinal fluid, submandibular gland, nasal mucosa and skin), skin biopsies are simplest to obtain with minimal risk. For skin aSyn SAA to be a scalable biomarker, optimization of preanalytical specimen acquisition and processing procedures is warranted. We recently showed that the sensitivity of aSyn SAA is superior in frozen skin biopsies over formalin-fixed tissues. This study aims to further improve the tissue retrieval process of formalin-fixed tissues and to directly compare the sensitivity and specificity of our aSyn SAA in frozen vs formalin-fixed skin biopsies from the same individuals with PD, those at-risk for it, or healthy controls.

Methods: In the Parkinson’s Progression Markers Initiative (PPMI) study, two 3-mm skin punch biopsies were collected on 25 early PD, 25 healthy controls and a small number of at-risk/prodromal cases from the cervical paravertebral region. One biopsy was placed in formalin, the other placed in saline followed by freezing. Each blinded sample was processed for analysis by aSyn SAA, with formalin-fixed samples undergoing our new KASAR protocol prior to published skin RT-QuIC assay.

Results: aSyn SAA parameters in frozen vs fixed specimens will be compared, including time-to-threshold and maximal fluorescence. Sensitivity/specificity for PD diagnosis will be calculated and compared with clinical findings.

Conclusions: The results will inform critical preanalytical aspects of skin biopsy acquisition and processing for aSyn SAA as a PD biomarker.
Aims: Parkinson’s disease (PD) is associated with a loss of central dopaminergic pathways in the brain leading to an abnormality of movement, including saccades. Saccadic eye movement parameters have been proposed as a neurophysiological biomarker for Parkinson’s disease but their potential as such remains controversial. We investigated the effects of dopaminergic medication on prosaccadic and antisaccadic eye movement parameters.

Methods: We studied saccades both “off” and “on” medication in 14 idiopathic PD patients excluding those with atypical parkinsonism or dementia. Five patients who did not perform all oculomotor tasks involved were excluded. All participants exhibited no eyelid opening apraxia or other clinically evident eye movement abnormalities. Latencies, amplitudes, velocities, and directional errors were evaluated, using a published standardised protocol.

Results: Prosaccadic latency was significantly prolonged by dopaminergic medication in PD (p< 0.001), from a mean of 256.85 ms in the “off” medication state to a mean of 303.64 ms in the “on” medication state. There was no effect of medication on antisaccade task parameters including error rate and latency.

Conclusions: Prosaccadic latency is significantly affected by dopaminergic medication which tends to make PD patients slower and may complicate its use as a biomarker in drug trials.
Aims: Parkinson's disease (PD) is a neurodegenerative disorder, displays misfolding of α-synuclein protein as a cardinal feature. At present, employing SPECT/PET scans clinically for diagnosis are widely used. However, about 70% neurons are damaged at the time of diagnosis, and the invasive nature of this method limits its utility. Our goal is to target the earliest misfolded form of this protein (β-cross sheet), enabling quick diagnosis of PD at an initial stage thereby preventing further neuronal damage. Therefore, the aim is to develop a polyclonal antibody specific to β-cross sheet of α-synuclein protein and investigate its interaction with different forms of α-synuclein.

Methods: Polyclonal antibody, produced by immunizing white New Zealand rabbits with β-cross sheet of α-synuclein, taken as immunogen, was emulsified with Freund’s complete adjuvant (FCA) and administered intradermally to the rabbits for primary immunization. Preparation of booster injections and its administration was identical to the primary injection, except that FCA was replaced with Freund’s Incomplete adjuvant (FIA). The activities of developed antibody were evaluated based on high antibody titre determined by dot blot method. The antibody was then partially purified by centrifugation, filtration and precipitation. Its sensitivity, selectivity and specificity with different forms of α-synuclein protein were assessed by dot blot analysis.

Results: The results demonstrated that the polyclonal antibody against β-cross sheet of α-synuclein protein was successfully produced and showed differential binding to different forms of antigen.

Conclusions: The results obtained so far indicate the specificity of the polyclonal antibody and its probable use in the early diagnosis of PD.
POSTERS

AUTONOMIC SYMPTOMS ARE REFLECTED IN AUTONOMIC FUNCTION TESTS AND ASSOCIATED WITH STRIATAL DOPAMINE TRANSPORTER ACTIVITY IN PARKINSON'S DISEASE

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\textbf{Aims:} Autonomic symptoms have major impacts on daily life of patients with Parkinson's disease (PD). The aim of this study is to investigate the correlation between objective autonomic measures and subjective autonomic symptoms in patients with PD.

\textbf{Methods:} Autonomic symptoms of patients were assessed by structured questionnaire (AsQ) and non-motor symptom scale (NMSS). All of 122 patients took dopamine transporter (DAT) PET using F-18-FPCIT, and autonomic function tests using composite autonomic severity score (CASS) and delayed heart-to-mediastinum ratio (HMR) from I-123 MIBG cardiac SPECT.

\textbf{Results:} Patients with PIGD phenotype showed characteristics of more advanced disease status and increased total score of AsQ than tremor-dominant phenotype. However there was no difference in CASS and HMR. Based on HMR, abnormal HMR group showed trend of higher AsQ and significantly increased CASS. According to CASS, normal CASS group was younger and showed milder disease status compared to other groups. Normal CASS group showed lower GI subscore of NMSS and AsQ, and higher HMR than other groups. Both of normal HMR group and normal CASS group showed significantly increased striatal DAT. Bivariate correlation analyses revealed correlation of GI subscore of NMSS and AsQ with HMR and CASS. Multiple regression analyses using age and disease duration as covariate confirmed association between HMR and sexual subscore of AsQ, and between CASS and AsQ.

\textbf{Conclusions:} The results of this study found that objective autonomic measures well reflected subjective autonomic symptoms, and also suggested association between symptoms and signs of autonomic dysfunction and striatal DAT activity in PD.
COMPARATIVE ANALYSIS OF DIFFERENT PATHOLOGICAL ALPHA-SYNUCLEIN MARKERS IN SKIN BIOPSY AS BIOMARKERS OF PARKINSON'S DISEASE AND ATYPICAL PARKINSONISM: A LONGITUDINAL STUDY

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Aims: Pathological α-synuclein (αSyn) and small fiber neuropathy (SFN) have been demonstrated by skin biopsy in Parkinson's disease (PD). In a longitudinal study, we evaluate the diagnostic and prognostic capacity of oligomeric, aggregated and phosphorylated αSyn, and SFN in PD and atypical parkinsonism (AP).

Methods: A 3mm punch skin biopsy was performed at cervical and ankle sites in 30 idiopathic PD, 22 age-matched healthy controls (HC), 12 multiple system atrophy (MSA), and 11 AP with tauopathies (AP-Tau). Skin sections were analysed for oligomeric αSyn by proximity ligation assay (αSyn-PLA), phosphorylated αSyn (P-αSyn), and aggregated αSyn by 5G4 antibody (αSyn-5G4). Intraepidermal nerve fiber density (IENFD) was assessed as a measure of SFN. 24 PD were reanalysed after two years (T24).

Results: Pathological αSyn was more expressed in PD and MSA. αSyn-PLA showed the highest diagnostic accuracy (PD vs. HC sensitivity 80%, specificity 77%; PD vs. AP-Tau sensitivity 80%, specificity 82%). SFN was detected in PD and MSA, and a progression of denervation, not of pathological αSyn, was seen in PD at follow-up. Lower IENFD at baseline was associated with cognitive and motor decline in PD. A skin biopsy-derived compound marker, resulting from a linear discrimination analysis model of αSyn-PLA, P-αSyn, αSyn-5G4, and IENFD, stratified patients with accuracy (77.8%), including the discrimination between PD and MSA (84.6%).

Conclusions: Skin biopsy is a comprehensive diagnostic tool for PD, where the choice of pathological αSyn marker and anatomical site significantly influences the diagnostic performance. Skin denervation, not pathological αSyn is a potential progression marker for PD.
ANALYSIS OF PRKN, PINK1, AND ZNF746 GENES AND THE LEVEL OF THEIR PROTEIN PRODUCT IN PATIENTS WITH PARKINSON'S DISEASE

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Aims: Objectives: Parkinson's disease (PD) is the second most common neurodegenerative disease. PD in most cases takes the sporadic form and is caused by environmental factors together with/or genetic predisposition (90%). A number of genes directly related to PD have also been identified, variants of which can lead to familial PD, accounting for 10% of all cases. The aim of this study was to analyze variants: PRKN (c.500G>A, c.520C>T, c.823C>T), PINK1 (c.1562A>C), and ZNF746 (c.159C>T) and to measure the plasma concentration of parkin and PINK1 proteins in PD patients and controls.

Methods: The study included above 80 individuals, PD patients, and controls. The genetic variants were analyzed by HRM, RT-PCR, and sequencing methods. The concentration of parkin and PINK1 proteins was determined using ELISA kits.

Results: Demonstrated in only one participant from PD patients who were a simultaneous carrier of c.500G>A and c.520C>T variant. In the case of the PINK1 analysis, the correct variant of AA was demonstrated in 31.6% of PD patients. It was almost two times lower percentage of respondents than in the controls (p<0.05). None of the PINK1 variants had a significant effect on the course of PD. The c.159C>T change in the ZNF746 gene was detected in one participant who belonged to controls. The parkin levels showed significantly lower values in the PD patients (p<0.05) than in controls. The level of PINK1 did not differ significantly between the study group and controls.

Conclusions: The data obtained may contribute to the improvement of the early diagnosis of PD.
A NOVEL PATHOGENIC SNCA MISSENSE VARIANT K58N ASSOCIATED WITH EARLY-ONSET PARKINSON’S DISEASE

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Aims: Monogenic forms of Parkinson’s disease (PD) due to SNCA mutations are rare and the majority consist of duplications and triplications of the gene. Only seven disease-causing point mutations in alpha-synuclein (aSyn), the protein encoded by the SNCA gene, have been described to date. The objective of this study was to characterize a novel pathogenic point mutation in SNCA.

Methods: Whole-exome-sequencing was performed on a patient with early-onset (age at onset: 38 years) tremor-dominant PD, whose father and grandfather were also affected, suggesting autosomal-dominant inheritance. In vitro studies with recombinant protein and aggregation assays in cell models of aSyn aggregation were also performed.

Results: The patient harboured the rare variant c.174G>C; p.K58N that is not annotated in gnomAD. The K58 residue exhibits a high phylogenetic conservation, and in-silico-algorithms predict a deleterious effect of the substitution K58N. In vitro studies showed that the K58N mutation alters the kinetics of the fibrilization reaction of aSyn, and that it affects the aggregation in cell models.

Conclusions: Our data suggests that K58N is a novel pathogenic variant and provides insight into the molecular effects of the mutation on the aggregation of aSyn. Ultimately, unravelling the molecular mechanisms modulating aSyn aggregation will be instrumental for the design of future strategies for therapeutic intervention.
PARKINSON’S DISEASE PATIENT WITH A HETEROZYGOUS SNCA DELETION: A CASE REPORT

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Aims: To describe a unique case of Parkinson’s disease (PD) patient with a heterozygous deletion of SNCA (encoding alpha-synuclein), along with analysis of alpha-synuclein levels in peripheral blood mononuclear cells (PBMCs) of the patient.

Methods: SNCA was sequenced using Molecular Inversion Probes (MIPs) and genotyped using OmniExpress + NeuroX microarray chip. ExomeDepth and PennCNV were used to detect copy number variations (CNVs) in MIPs and microarray data, respectively. We used Multiplex-ligation dependent probe amplification to validate CNVs. Alpha-synuclein protein levels were also examined in PBMCs of the patient of interest as well as six other PD patients and seven healthy controls. Different antibodies targeting different epitopes of alpha-synuclein were used.

Results: The patient was of French-Canadian descent and was diagnosed with idiopathic PD at age 71. The patient carried a 20.4Mbp heterozygous deletion from rs7681742 to rs7670522 (chr4:85730752-106160365, hg19) including SNCA. No other PD-related variants, nor CNVs were found in GBA, SNCA, VPS35, GCH1, PRKN, LRRK2, PINK1, PARK7. Interestingly, alpha-synuclein was detected in PBMCs of other PD patients and controls, whereas no alpha-synuclein protein expression was found in the patient with the SNCA deletion.

Conclusions: This study describes a patient with reduced amount of alpha-synuclein, which was not protective for PD in this specific case. Whether the current therapeutic approach of reducing alpha-synuclein levels will benefit PD patients remains to be further studied.
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POSTERS

LACK OF EPISTATIC INTERACTION OF ALPHA-SYNUCLEIN WITH APOE IN SYNUCLEINOPATHIES PARKINSON’S DISEASE, DLB AND IRBD

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Aims: Two recent studies suggested that the €4 haplotype in APOE was associated with increased α-synuclein pathology in cell and mouse models. Genetic variants in the SNCA region have strong association with Parkinson’s disease (PD), Dementia with Lewy Bodies (DLB), and idiopathic REM Sleep Behavior Disorder (iRBD), while APOE is a genetic risk determinant for only DLB. To determine if genetic-level interactions between SNCA and APOE exists that can explain the protein-level association, we
investigated the genotypic interaction of APOE and SNCA in cohorts of PD, DLB, and iRBD.

**Methods:** We analyzed genome-wide association study (GWAS) data from 5,229 PD patients and 5,480 controls, 2,610 DLB patients and 1,920 controls, and 1,223 iRBD patients and 3,627 controls. We used logistic regression interaction models across all 3 cohorts independently between the 1) top GWAS signals of SNCA SNPs and APOE haplotypes, 2) SNP x SNP and 3-way SNP interaction across the entire coding region plus 200kb flanking each gene.

**Results:** No significant interactions were found to be statistically significant after correction for multiple testing across all three cohorts in both sets of analyses.

**Conclusions:** Our results do not support a role for genetic interactions between APOE and SNCA across PD, DLB, and iRBD. Since the tested genetic variants affect the expression and function of these proteins, it is likely that any interactions between them does not affect the risk of PD, DLB and iRBD.
POSTERS

DEVELOPING NEW INSIGHTS INTO CLINICAL AND GENETIC FEATURES OF PD - THE GLOBAL PARKINSON'S GENETICS PROGRAM (GP2) CLINICAL COHORTS WORKING GROUP

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Aims: To recruit Parkinson's cohorts across the world for the the Global Parkinson's Genetics Program (GP2, http://gp2.org/), harmonise clinical data for joint analysis and ensure that data can be shared and used within the GP2 network. Our overall aim is to integrate clinical and genetic data from 150,000 participants worldwide in a collaborative project.

Methods: We have established a pathway for identifying and evaluating clinical cohorts from different contexts including brain banks, drug trials and longitudinal studies. For data harmonisation we have established a set of core clinical data in a common data format to allow data harmonisation. The incoming data from each cohort is re-coded into a standard data format. Quality control is performed in collaboration with the cohort investigators to ensure continuity.

Results: Over 80 PIs/cohorts have joined the study so far representing over 75,000 PD patients. We have developed a harmonised clinical data template and a process for standardising clinical data. Genotyping using the newly designed Neurobooster chip is underway and we anticipate case-control and genotype-phenotype results through 2022. We are collating clinical and biosample data and preparing for rapid data release.

Conclusions: The GP2 project is enabling collaborative research into the genetics of PD at a dramatically increased scale, and with enhanced scope for collaboration. This will lead to new insights into the biology and potential future treatments for PD.
COMMON GENETIC VARIATION IN THE CLINICAL PROGRESSION TO PARKINSON'S DISEASE DEMENTIA

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Aims: The rate of disease progression and severity are highly variable among people with Parkinson’s disease (PD), but the contribution of genetic factors to clinical heterogeneity is still incompletely understood. Our aim is to expand on previous work to investigate genetic factors associated with progression to PD dementia (PDD).

Methods: Imputed SNP array and whole-genome sequence data from 3840 PD cases from four independent longitudinal cohorts were used to perform genome-wide and candidate-loci time-to-event analysis, using dementia as the endpoint. Time-to-event was calculated from disease onset or diagnosis until the detection of dementia.

Results: The incidence of dementia across cohorts was 7.0% and the median time to dementia onset was 6.1 years. The top SNP on the meta-analysis of cohort-specific time-to-event GWAS identified a significant association with the ε4-tagging APOE variant rs429358, indicating that ε4 carrier status is a strong determinant of dementia in PD (HR = 2.45 , P = 1.757x10^-14). This was confirmed in the candidate-loci time-to-event analysis of the combined cohorts, which in addition identified a strong association of progression to PDD in GBA mutation carriers (HR = 2.78, P = 4.22x10^-6). Other associations recently reported have not been confirmed.

Conclusions: Findings from this study confirm APOE and GBA as significant risk factors for progression to PDD, while not corroborating other associations described in the literature. This highlights the need for larger and better characterised longitudinal patient cohorts to overcome heterogeneity in study design and data collection, as well as to detect variants with smaller effect sizes.
GENOME-WIDE ASSOCIATION STUDY OF EARLIER ONSET DEMENTIA IN LEWY BODY DISEASE

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Aims: Up to 80% of PD patients develop dementia (PDD), but time to dementia varies widely from motor symptoms onset. Dementia with Lewy bodies (DLB) presents with clinical features similar to PDD, but cognitive impairment precedes or coincides with motor impairment onset. It remains controversial whether DLB and PDD are distinct conditions or represent part of a disease spectrum. The biological mechanisms underlying disease heterogeneity, in particular the timing of dementia, remain poorly understood, but will likely be key to understanding disease pathways and ultimately therapy development. Previous genome-wide association studies (GWAS) in PD and DLB/PDD have identified risk loci differentiating patients from controls. Here, we will complete a GWAS comparing PD to DLB/PDD to decode disease heterogeneity by investigating the genetic drivers of earlier dementia onset in Lewy Body Diseases.

Methods: We collated data for 7,712 patients of European ancestry from PRoBaND, OPDC, and AMP-PD. We defined “Lewy body accelerated dementia (LBAD)” patients as either having a diagnosis of DLB or PDD within 5 years of symptoms onset. We conducted a discrete phenotype GWAS comparing LBAD vs. PD.

Results: We found significant association with accelerated dementia at rs429358 on chromosome 19 and rs6841352 on chromosome 4. rs429358 is the APOE e4 tagging variant (p = 1.37E-57, OR = 2.87). rs6841352 (p = 1.02E-12, OR = 1.43) is in high LD with the lead SNP rs7680557 in LBD GWAS (Chia et al., 2021), which is in eQTL with SNCA-AS1.

Conclusions: We plan to conduct post-GWAS analysis to further investigate the downstream impact of these variations.
POSTERS

ASSESSMENT OF PARKINSONIAN SYMPTOMS AND TOXIN EXPOSURES IN FIREFIGHTERS

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Aims: The purpose of this study is to improve our understanding of the relationship between toxin exposure in firefighters and Parkinsonian symptoms. Parkinson’s disease (PD) has been correlated with several environmental exposures. The frequency of PD in firefighters is higher than the general population, which may be due to the toxin exposures firefighters experience on the job. There is a need to address the high rates of PD among this subgroup.

Methods: An anonymous survey distributed to Massachusetts firefighters assessed risk factors for toxin exposure and presence of Parkinsonian symptoms. Risk factors included frequency and duration of time spent firefighting, number of 5-9 alarm fires worked, and history of toxin exposure (i.e. pesticides). We collected the frequency of Parkinsonian symptoms including tremors, muscle stiffness, REM behavior disorder, hyposmia, micrographia, and decreased walking pace. Analyses comparing toxin exposure and presence of Parkinsonian symptoms were performed using Chi-square testing, p < 0.05 was considered significant.

Results: Two hundred participants were included in the study. The number of years as a firefighter, the number of days per week working as a firefighter, and the number of 5-9 alarm fires worked correlated with higher reports of hyposmia, micrographia, and decreased walking pace using Chi-square testing with p values < 0.05.

Conclusions: Our study showed that the number of years working as a firefighter, the number of days per week working as a firefighter, and the number of 5-9 alarm fires worked correlated with higher reports of Parkinsonian symptoms such as hyposmia, micrographia, and decreased walking pace.
Aims: Predictors of future PD have been suggested through population-based studies, although these studies over-represent white, affluent groups and may not be generalisable.

Methods: A case-control study was conducted in East London, using primary care health records. Logistic regression was used to determine associations between risk factors and pre-diagnostic presentations with PD diagnosis. Three periods (recorded <2 years, 2-5 years, and 5-10 years prior to diagnosis) were analysed.

Results: Primary care records were available for 1,055 PD patients and 1,009,523 controls. The strongest associations were found for tremor (odds ratio [OR], 181.69; 95% confidence interval [CI], 151.9-217.31) and ‘memory complaints’ (OR, 9.84; 95% CI, 7.39-13.11), both at 0 to <2 years before PD diagnosis. Tremor still showed a strong association with subsequent PD 5-10 years prior to diagnosis (OR, 14.61; 95% CI, 9.71-21.98). Shoulder pain was more common in those who developed PD 5-10 years later compared to those who did not (OR, 2.54; 95% CI, 1.77-3.65) and may be a surrogate marker for rigidity, which had low prevalence (1.2%). Of comorbidities, epilepsy showed the highest association with future PD (OR, 5.14; 95% CI, 1.26-21.0). Associations were found for hypertension (OR, 1.71; 95% CI, 1.34-2.17) and type 2 diabetes (OR, 1.57; 95% CI, 1.31-1.87) 5-10 years before PD diagnosis. No associations with PD diagnosis were found for ethnic group or deprivation index.

Conclusions: Comorbidities and PD manifestations are commonly reported in primary care prior to PD diagnosis in a highly diverse, generally deprived population with universal access to health care.
GP2: TRAINING THE NEXT GENERATION OF PARKINSON’S DISEASE GENETICS RESEARCHERS - ALL OVER THE WORLD

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Aims: To establish a virtual ‘center of excellence’ with resources and expertise to serve the training needs of the Global Parkinson’s Genetics Program (GP2; www.gp2.org) and its collaborators.

Methods: The training, networking and communication group was established at the beginning of GP2. Training was broadly divided into training for ‘individuals’ and ‘groups’ to achieve breadth and impact. For groups, we developed a free and accessible web-based learning platform (https://training.gp2.org/) to establish foundational knowledge of PD genetics and related topics. For individuals, tailored research training opportunities (from short courses to master's and PhD programs) have been created, prioritising clinicians, scientists and researchers from traditionally underrepresented regions in research.

Results: To date, web-based courses in bioinformatics, Terra and PD genetics have been launched with more than 325 current learners. Trainees have been supported to attend graduate courses in bioinformatics and data science at the Foundation for Advanced Education in the Sciences at the NIH. PhD and master’s level training in Africa, Asia and Latin America is now starting. A trainee network has been created to streamline opportunities, direct expertise to the places where it is needed, and to facilitate access to data and analysis across GP2, with 85 members from around the world.

Conclusions: Training the next generation of PD researchers worldwide is a priority for GP2. Over the coming years, our reach will expand to ensure that needs are met and research capacity is generated where it is needed to further our understanding of the genetic basis of PD.
Aims: PREDICT-PD is an online, population-based, cohort study aiming to identify people in the prodromes of PD. Here, we describe recruitment to the PREDICT-PD study, along with sharing insights about methods of recruitment to inform similar studies.

Methods: The pilot phase of PREDICT-PD began in April 2011 with an initial sample size of 1323 healthy volunteers aged 60-80 years. Participants that remained under follow-up were transferred to the next phase of PREDICT-PD in December 2018 and recruitment re-opened with a target sample size of 10,000. Over three years we have utilised a variety of methods to advertise PREDICT-PD and drive recruitment. These included advertising to Parkinson's and dementia-related research networks, independent members groups, news articles, radio shows, and most recently, via automated screening of eligible participants from primary care records. For the latter, eligible individuals were sent an SMS from their primary care physician inviting them to participate.

Results: At the time of writing, 8566 participants had registered with PREDICT-PD and 4527 participants had consented to and completed baseline assessments. Recruitment was divided into three phases: December 2018-January 2020 (pre-pandemic), January 2020-June 2021 (first and second waves of the UK pandemic), and June 2021-present. Registrations averaged 350-400, 50-100 and 150-200 participants per month, respectively, in these three periods.

Conclusions: Temporal trends in recruitment may be determined by the target population (e.g. use of research registries or not), external events (e.g. COVID pandemic) and method of recruitment (passive versus active). These observations may help recruitment strategies now and for future studies.
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Aims: α-Synuclein (αSyn) aggregation in Lewy bodies/neurites defines familial and ‘sporadic’ Parkinson's disease. We previously identified α-helical αSyn tetramers, in addition to monomers, in normal cells. PD-causing αSyn mutations decrease the tetramer:monomer (T:M) ratio, associated with αSyn hyperphosphorylation and cytotoxicity in neurons and a motor syndrome of tremor and gait deficits in transgenic mice. We asked whether LRRK2 mutations, the most common genetic cause of cases previously considered sporadic PD, also alter αSyn tetramer homeostasis.

Methods: We used induced pluripotent stem cell (iPSC) technology to study human neurons derived from PD patients carrying LRRK2 mutations in either its GTPase domain (R1441C) or its kinase domain (G2019S). We used the cell-penetrant crosslinker DSG to trap physiologic, multimeric assemblies of αSyn and visualized them by immunoblotting to derive their T:M ratios. We correlated the multimeric species with pSer129 phosphorylation levels of αSyn as an additional readout for αSyn-related pathology. We compared both readouts with their isogenically-corrected lines to exclude cell line-specific confounders.

Results: Patient neurons carrying G2019S, the most prevalent mutation, or R1441C each had significantly decreased T:M ratios and pSer129 hyperphosphorylation. Two LRRK2 kinase inhibitors normalized T:M ratio and hyperphosphorylation in G2019S but not R1441C mutants. An inhibitor of stearoyl-CoA desaturase, the rate-limiting enzyme for monounsaturated fatty acid synthesis, restored T:M ratios in both mutants.

Conclusions: With the discovery that PD-causing mutations of glucocerebrosidase in Gaucher's carriers also decrease T:M ratios, our findings suggest that three distinct genetic forms of PD involve life-long destabilization of αSyn tetramers as a common pathogenic mechanism.
ROLE OF SNCA-AS1, THE ALPHA-SYNUCLEIN ANTISENSE TRANSCRIPT IN PARKINSON’S DISEASE: DISRUPTION IN SYNAPTIC PROCESSES AND ALPHA-SYNUCLEIN MODULATION

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Aims: This work aims to characterize how SNCA-AS1, antisense transcript to the SNCA gene, affects cellular processes, PD pathogenesis and alpha-synuclein (alpha-syn) biology.

Methods: SH-SY5Y cells stably transfected with SNCA-AS1 were subjected to RNA-sequencing to investigate its impact on gene expression. RNA interference was used to inhibit SNCA-AS1 expression. Real Time-PCR and western blot were used to verify SNCA-AS1’s effect on SNCA’s expression, and synapses-related markers. RNA-pull down was used to verify SNCA-AS1 and SNCA/alpha-syn interaction. SNCA half-life was assessed via Actinomycin D treatment. SH-SY5Y cells were treated with SNCA-AS1 overexpressing cells supernatant to assess alpha-syn uptake.

Results: The overexpression of SNCA-AS1 upregulates SNCA mRNA and protein, whilst its inhibition down-regulates both targets. This appears to strongly impact neurite extension and synapses’ biology, through specific molecular signatures. Following SNCA-AS1 over-expression, we report a reduced expression of markers associated with synaptic plasticity, and we specifically focus on GABAergic and dopaminergic synapses, for their relevance in aging processes and PD, respectively. We found that the opposite upholds when SNCA-AS1 is down-regulated. Moreover, the upregulation of SNCA-AS1 leads to alterations in numerous PD specific genes (e.g. VMAT and DRD2). We report that SNCA-AS1 directly binds SNCA but not alpha-syn protein and we demonstrate that SNCA-AS1 overexpression impacts on the decay’s kinetic of SNCA mRNA. Lastly, we report that SNCA-AS1 overexpressing cells release alpha-syn and lead to an increase of its uptake by target cells.

Conclusions: Our results show that SNCA-AS1 elicits its cellular functions impacting alpha-synuclein biology, synapses biology and PD-related genes.
SNCA SILENCING REVEALS NOVEL SEX-SPECIFIC ALPHA-SYNUCLEIN FUNCTIONS IN MICE

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Aims: Alzheimer’s disease and Parkinson’s disease, two of the most common neurodegenerative disorders, might share signaling pathways that could account for common pathology and symptomology. We previously described how genetically ablating SNCA encoding alpha-synuclein in a mouse model of Alzheimer’s improved spatial memory. Antisense oligonucleotides (ASOs) are a clinically relevant method of transiently lowering target gene transcripts. We investigated whether treatment with ASO targeting murine SNCA mRNA improved cognition in mice overexpressing human amyloid precursor protein.

Methods: Male and female mice were injected with ASO⁹⁹⁹¹ and learning and memory were tested using the Barnes circular maze. SNCA knock-out (SNCA-KO) mice were tested on the same behavioral paradigm, and brain tissue was collected for NanoString transcriptomic analysis, confocal microscopy and biochemistry.

Results: Administration of the murine ASO⁹⁹⁹¹ lowered both SNCA mRNA and protein, and improved cognition in male, but not female mice. To evaluate potential differences in αSyn function across sexes, we used SNCA-KO mice. Surprisingly, constitutive ablation of SNCA worsened spatial memory in female, but not male mice. Transcriptomic analysis revealed differentially regulated genes related to synaptic signaling, microglia, interneurons, mitochondria and the Ras signaling pathway. Notably, EGR1, a transcription factor involved in synaptic plasticity and neuronal activity regulation, and several EGR1 target genes were upregulated in female animals compared to males.

Conclusions: These results demonstrate that αSyn function differs between male and female mice. Thus, this novel finding should be considered when designing translational studies.
Aims: We aimed to delineate the specific patterns of hypometabolism (cortical and subcortical) and DAT bindings, cardiac MIBG and HRV that are characteristic of PD with Glucocerebrosidase (PD-GBA) compared with sporadic PD (sPD) using quantitative analyzing method.

Methods: We investigated 17 of PD-GBA and 24 sPD patients. GBA-related PD group and sporadic PD group were compared regarding clinical, FP CIT-PET, cardiac autonomic function (Cardiac MIBG, HRV, HUT). FP-CIT PET was performed with dual-phase method, to gain both early-phase (brain metabolism) and delayed phase (DAT). The PET imaging data was analyzed by quantitative analysis using PMOD software.

Results:

PD-GBA group had more common RBD, OH and neuropsychiatric symptoms. Early CIT-PET showed significant lower SUVR on bilateral parietal (0.943±0.054 vs. 1.015±0.044, p<0.001) and occipital lobes (0.988±0.062 vs. 1.040±0.058, p=0.043). The delayed CIT-PET imaging revealed slightly lower SUVR
values on bilateral putamen on GBA-PD group than IPD group (2.903±0.710 vs. 3.493±0.713, p=0.022) with similar asymmetry and caudate-to-putamen ratio. HRV results are similar between groups. In addition, PD-GBA had more frequent abnormal cardiac MIBG findings (H/M ratio < 1.7, 72.8% vs 31.6%, p=0.029).

**Conclusions**: This study is the first investigation to compare comprehensive dual CIT-PET and Cardiac MIBG between GBA-PD and sPD. While DAT binding is similar, there was significant lower uptake in bilateral posterior parietal and occipital cortex in patients with GBA-PD. Cardiac autonomic function test (MIBG, HRV) revealed more severe involvement in GBA-PD. These findings suggest that GBA mutation could accelerate a-synuclein aggregation in PD, particularly predisposing body-first PD subtype.
POSTERS

PHARMACOLOGICAL INHIBITION OF LYSOSOMAL GLUCOCEREBROSIDASE ACTIVITY IN DOPAMINERGIC LUHMES CELLS

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Aims: Loss-of-function mutations in the GBA gene encoding lysosomal glucocerebrosidase are common genetic factor for Parkinson’s disease. However, how glucocerebrosidase defects contribute to Parkinson’s disease pathophysiology is not elucidated. The present study aims to explore how a decrease in lysosomal glucocerebrosidase activity impacts on dopaminergic cell susceptibility to different pathways of neuronal death.

Methods: Conduritol-beta-epoxide (CBE) was used to inhibit glucocerebrosidase activity in immortalized Human dopaminergic LUHMES cells exposed to various cell-death inductors including staurosporine, rapamycin, erastin, RSL3, MPP+, rotenone leading to apoptosis, autophagy, ferroptosis or death by mitochondrial respiratory chain alteration, respectively. LUHMES cell viability and reactive oxygen species production were assessed using resazurin and flow cytometry.

Results: Glucocerebrosidase short-term inhibition of 1h, 24h, 48h, 72h or 96h didn’t increase LUHMES cells susceptibility to inductors of apoptosis, ferroptosis and autophagy-induced neuronal death. Future experiments will aim (i) to assess LUHMES cell viability and oxidative stress in long-term inhibition of glucocerebrosidase, (ii) characterize the pro-inflammatory profile induced by CBE compared to LPS, in the Human microglial HMC3 cell line and (ii) to assess CBE-induced microglial activation could cause oxidative stress and neuronal death.

Conclusions: Our data show that loss of glucocerebrosidase activity does not directly sensitize dopaminergic neurons to death but that this may be mediated by microglia-dependent pathways.
POSTERS

AGE- AND SEX-SPECIFIC GENE EXPRESSION ALTERATIONS IN PARK7-/- MOUSE MODEL

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Aims: We aim to characterize the gene regulatory networks underlying transcriptomic changes in prodromal stages of Parkinson’s disease (PD) to facilitate earlier and more accurate diagnosis. Through an integrative analysis, we aim to identify the upstream regulatory events and key transcription factors controlling pathogenic gene expression changes at chromatin level.

Methods: We have investigated the gene expression signatures and chromatin profiles during aging of in vivo midbrain sections and isolated nuclei of different cell types from a knock-out mouse model for Park7, a gene often mutated in early-onset PD.

Results: We have observed a robust age and sex-dependent transcriptomic deregulation in these mice. Astrocytes, a cell population providing support to the dopaminergic neurons and protecting them against oxidative stress, seem to play an important role in these alterations. Some of the changes are shared with a mouse model overexpressing human A30P mutated alpha-synuclein transgene. In addition, some of the changes can be observed also in vitro in cell types differentiated from PARK7 PD patient-derived induced pluripotent stem cells.

Conclusions: Our findings in Park7-/- mouse model, pointing out astrocytes as important players in neurodegeneration, may help a better understanding of the disease and pave way towards an earlier diagnosis. Some of the transcriptomic changes are shared with other PD mouse models and with a human in vitro model of PARK7-linked PD, supporting the relevance of our finding for PD. Further experiments to validate the upstream regulators and identified pathways are currently in progress.
BRIEF IN-SILICO STUDY OF PATHOGENIC VARIANTS OF PLA2G6

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Aims: Mutations in the Phospholipase A2 Group 6 (PLA2G6) gene cause neurodegenerative disorders, possibly due to their effects on membrane homeostasis, thus damaging the cells. We tried to predict the functional and structural impact of six rare variants of PLA2G6 in our centre- chr22:38509598 G>A, chr22:38508565 C>T, chr22:38508566 G>A, chr22:38525518 C>A, chr22:38541520 T>C, chr22:38536020 T>C using computational analysis tools, and thus explore the phenotype-genotype correlation. These variants had been detected in people with Parkinson's-dementia complex, and in others who had schizophrenia.

Methods: The functional analysis of the variants was done using MutationAssessor, SIFT, PROVEAN, PolyPhen 2, and Align GVGD, MUpro and I-Mutant Suite. The 3D structure of human PLA2G6 protein was predicted by RaptorX and modelled using UCSF Chimera to compare the native and the mutant amino acids.

Results: The functional analysis classified all the variants as deleterious, in varying degrees.

Conclusions: Many individuals had developed severe parkinsonian side-effects when treated with antipsychotics as they also had psychological symptoms. Exposure to antipsychotics in these patients might have precipitated symptoms of PD. It is known that PLA2G6 expression is significantly decreased by antipsychotic drugs. This additional inhibition, in the presence of an already altered protein structure/function in those that carry the mutation, would have greatly amplified the risk of Parkinsonism. Distinct phenotypes, including the propensity for adverse drug reactions, may be associated with mutations in the same gene. This may need further exploration, in addition to studying the influence of additional genetic and environmental factors.
Aims: Parkin confers protection in the ageing human brain. We recently discovered that parkin can function as antioxidant. There, we demonstrated that parkin neutralizes reactive oxygen species and sequesters reactive electrophilic species including dopamine radicals. Ex vivo, parkin also augments melamin polymer formation from dopamine (Tokarew et al., 2021). Here, we explored whether parkin is involved in neuromelanin formation in the human midbrain.

Methods: We visualized parkin localization within pigmented dopaminergic neurons in the Substantia nigra (SN) in post mortem tissue from human control midbrain using immunoelectron microscopy (IEM; n=2, ages 69 and 86 years) and immunofluorescence microscopy (n=17 cases, ages 2-85 years). We employed epitope-mapped, murine monoclonal antibodies to parkin (clones -B, -D, -E and -G; BioLegend), which had been characterized for their specificity by proteomics and routine histochemistry. IEM and immunofluorescence staining of SN tissues were performed, as published (Zucca et al., 2018; Tokarew et al., 2021).

Results: Sections of SN neurons from control subjects revealed extensive parkin reactivity within intracellular neuromelanin-containing organelles (within the pigment portion and lipid bodies of organelles) and LAMP3/CD63-positive lysosomes by both immunofluorescence and IEM. To a lesser extent, other organelles, such as the nucleus and perinuclear structures, likely representing endoplasmic reticulum and mitochondria, were identified as anti-parkin-positive by IEM.

Conclusions: Dopaminergic neurons of the SN from adult control subjects reveal extensive labeling of neuromelanin pigment by anti-parkin antibodies. We postulate that in adult human brain parkin participates in the processing and sequestration of dopamine radicals within neuromelanin-containing organelles, often within LAMP3/CD63-positive lysosomes.
Coevolution study of Tau and Alpha-synuclein suggests a connection between their normal interaction in neurons and the Parkinson's disease-associated mutation A53T

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Aims: The microtubule-associated protein tau and α-synuclein interact in vitro, and α-synuclein can also compete with tau binding to microtubules. To test whether these interactions might be part of their natural biological functions, a correlated mutation analysis was performed between tau and α-synuclein, looking for evidence of coevolution. Analyses including neuronal tubulin proteins and β- and γ-synuclein were also included.

Methods: A mutual information based correlated mutation analysis was employed to identify similar mutation patterns in multiple sequence alignments of the tau, synuclein and tubulin proteins across all vertebrate species. Hemoglobin beta chain was used as a negative control.

Results: Potential correlated mutations were detected between tau and α-synuclein, one involving an α-synuclein residue known to interact with tau in vitro, Asn122, and others involving the Parkinson’s disease-associated mutation A53T. No significant correlated mutations were seen between tau and β- and γ-synuclein. Tau showed potential correlated mutations with the neuron-specific βIII-tubulin protein. No convincing correlated mutations were seen between α-synuclein and the tubulin proteins.

Conclusions: The correlated mutations between tau and α-synuclein provide evidence suggesting the two proteins interact in vivo as part of their normal biological function in neurons. The involvement of the A53T mutation hints that their interaction might also play a role in the synucleinopathy of Parkinson's disease.
Aims: This study aimed to describe the differences in the proportions of circRNAs in the blood of Parkinson’s disease.

Methods: We called circRNAs from blood RNA-seq data from Parkinson’s Progression Marker Initiative (PPMI) corresponding to the baseline visit. We only included European samples (495 PD cases and 136 controls) for differential expression analyses (DE) to identify circRNAs that are significantly different between PD cases and controls (BH-adjusted p-values<0.05). Then, medication information was included in the model to investigate their role in the dysregulation of circRNAs.

Results: We identified nine circRNAs differentially expressed between cases and controls. When we added medication to the analyses, five of them remained significant, suggesting that those are dysregulated due to disease (circAFF2, circERBB2, circMYO9B, circSPI1, circZNF516); and the rest might be due to the medications (circFNDC3B, circPRRC2C, circSLAIN2, circTET2). In fact, the nine circRNAs showed similar patterns, increasing number of counts in relation to status (control<prodromal<PD). We also observed patterns related to the mutation in mutation-carriers, overall with higher counts compared to idiopathic PD.

Conclusions: Our findings suggest that some circRNAs are dysregulated in the context of PD. In fact, they seem to be dysregulated in a lesser extent during the prodromal phases of the disease. We identified nine circRNAs that were over-expressed in PD cases, especially for the mutation carriers, which we hypothesize that might be related to PD severity. We are replicating these findings using an independent dataset (Parkinson's Disease Biomarker Program), and performing longitudinal analyses using the remaining visits from PPMI.
LONGITUDINAL TRANSCRIPTOMIC AND FUNCTIONAL ANALYSIS OF PUTATIVE NEUROPROTECTIVE PATHWAYS IN A MOUSE MODEL FOR PARKINSON’S DISEASE

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Aims: Engrailed 1 (En1) is a highly conserved homeodomain transcription factor involved in programming and maintenance of midbrain dopaminergic neurons. Heterozygosity of En1 in SwissOF1 mice results in parkinsonian phenotype with progressive nigrostriatal degeneration. However, this phenotype is absent on C57Bl/6-En1+/- mice. The objective of this study is to characterize transcriptomic profiles of nigral dopaminergic neurons of SwissOF1 and C57Bl/6 wild-type and En1+/- mice to identify neuroprotective pathways.

Methods: Using histology and stereology, we assessed dopaminergic neurodegeneration, axonal swelling and dopaminergic innervation in the substantia nigra pars compacta and striatum of 4 and 16 weeks-old SwissOF1 and C57Bl/6 mice, both wild type and En1+/- . We further used RNAseq to analyze differential gene expression in this region at 1 week of age. Finally with functional enrichment and pathway reconstruction analysis we inferred involved signaling cascades.

Results: SwissOF1-En1+/- mice show a 23% loss of dopaminergic neurons at 16 weeks of age. No loss was detected on C57Bl/6-En1+/- mice. The transcriptomic data showed 134 differentially expressed genes between SwissOF1-En1+/- and SwissOF1-WT. In contrast, En1-hemizygosity induced differential expression in only 56 genes on the C57Bl/6 genetic background. Eleven enriched GO terms relevant to neuronal function, development, maintenance, neurodegeneration and/or neuroprotection were identified.

Conclusions: SwissOF1-En+/- mice develop a progressive and spontaneous PD-like phenotype which is absent in C57Bl/6-En+/- mice. With transcriptomics and GO enrichment we identified putative pathways responsible for the observed dopaminergic neuroprotection conferred by the C57Bl/6 background genome. Further contrasting the generated data to human cohorts data will provide valuable insight into novel approaches towards PD treatment.
Aims: In this study we applied untargeted metabolomic analysis to compare Parkinson's disease (PD) and essential tremor (ET), two of the most common neurologic conditions that often afflict older adults with tremor. Although clinical diagnosis is often clear, a subset of patients have overlapping features that make certain diagnosis difficult. Here, we utilized samples from PD and ET patients to determine whether metabolomic profiles of biofluids—CSF and blood—could distinguish these two conditions.

Methods: Patients undergoing deep brain stimulation surgery in Parkinson's disease (n=11) and essential tremor (n=7) at University of Kentucky were approached and consented under an IRB-approved protocol. Cerebral CSF and peripheral blood was collected during the operative procedure and immediately processed by centrifugation. Circulating metabolites in plasma and CSF were analyzed using gas chromatography-mass spectroscopy.

Results: There was alteration in multiple pathways which distinguished PD from ET in both CSF and plasma. Plasma metabolites that were significantly altered in patients with PD vs ET included sedoheptulose, malic and lactic acid, and eicosapentaenoic acid. In CSF, there was a substantial increase in beta-hydroxyisovalerate. PD plasma demonstrated distinct pentose phosphate pathway alterations, with elevation of NADPH, eNAMPT and NADH in plasma as confirmed by biochemical assays. α-Synuclein in plasma and CSF was higher in PD than ET.

Conclusions: Our results suggest that PD and ET have very different metabolic profiles that can be readily detected in biofluids, although verification with a larger dataset is required. Differentiation of PD and ET may be possible through utilization of a carefully selected set of plasma-based markers.
Aims: Parkinson’s disease (PD) is the second most prevalent neurodegenerative disorder in the ageing population. PD is characterized by the progressive loss of midbrain dopaminergic neurons (mDA) in the substantia nigra and by the accumulation of alpha-synuclein (α-Syn) in surviving neurons. Mutations in the SNCA gene that encodes α-Syn have been implicated in familial PD, but the precise contribution of these mutations to the PD phenotype remains unclear.

Methods: Here, we generated mDA neurons through differentiation of human induced pluripotent stem cells (hiPSCs) carrying the A53T mutation within the SNCA gene (SNCA-A53T) and a control line. We then employed single-cell RNA sequencing to investigate gene expression dynamics at several timepoints during mDA development and maturation. To investigate how mutation driven transcriptomic signatures manifested in the neuronal phenotype, bulk proteomics and image analysis were performed.

Results: Our preliminary data suggests that SNCA-A53T mutation induced transcriptional changes already manifests the PD phenotype during early neuronal differentiation. These molecular findings were complemented by the characterization of functional deficits in calcium homeostasis and mitochondrial dynamics.

Conclusions: Our results provide comprehensive single-cell data on SNCA-A53T specific transcriptomic modifications and support the contribution of the SNCA-A53T mutation in the pathogenesis of PD. Our study also addresses previously unresolved cellular heterogeneity associated with personalized PD iPSC models, by characterizing cell variability at a single-cell resolution. This study therefore represents a dynamic model of cell differentiation, able to recapitulate neuronal development and PD in vitro.
Aims: Multiple system atrophy (MSA) is a rare progressive neurodegenerative disorder pathologically characterized by the presence of α-synuclein aggregates in oligodendrocytes, termed as glial cytoplasmic inclusions. Accelerated epigenetic ageing has been reported for multiple tissues in several neurodegenerative diseases. To identify if patients with MSA exhibit accelerated brain ageing, we estimated the DNA methylation (DNAm) age as a surrogate for biological age in MSA cases and controls.

Methods: We used two DNA methylation-based clocks, Horvath’s multitissue clock and a recent cortical clock developed specifically for human cortical tissues. In dataset 1, samples consisted of white matter tissue from frontal and occipital lobes, and cerebellum. Additionally, a publicly available dataset (GSE143157, dataset 2) comprising prefrontal cortex grey matter was also analysed. DNAm age acceleration was calculated as: 1) difference between the predicted DNAm age and chronological age, and 2) residuals obtained by regressing DNAm age on chronological age and other confounders.

Results: Stronger positive correlations were observed between DNAm and chronological ages for the cortical regions using the cortical clock. However, for the cerebellar regions, the strongest positive correlation was observed with the Horvath’s multitissue clock. An age acceleration difference was observed in MSA cases compared to controls; however, upon adjustment for possible confounders (e.g., age, duplicate individuals, and neuronal proportions) the significance in age acceleration difference was lost.

Conclusions: DNA methylation clocks are useful tools in predicting the age of the patients, even in white matter tissue. However, our results highlight the need to account for confounding factors.
Aims: Having reliable biomarkers for Parkinson’s Disease (PD) is of clear importance. CpG DNA methylation data is one approach to identify individuals with PD. One frequent issue is that methylation data from different tissues have different methylation profiles making the analysis more complex. The objective is to obtain a technique that can identify patients with PD using CpG DNA methylation data from different tissues.

Methods: DNA Methylation data was obtained for 80 patients (40 control patients). Half of the samples were blood samples and the other half brain matter. Neural networks were used as a classification tool. The data was randomly divided into a training and testing datasets (each with 50% of the samples). Each patient had 484,211 CpG DNA methylation data analyzed. The neural network consisted on three layers (one hidden). The hidden layer contained 100 artificial neurons. The training dataset was used to train the algorithm and then the testing dataset was used to measure the accuracy of the results (the testing dataset was not used during the training phase).

Results: As shown in figure 1 (confusion matrix) the presented approach generates rather accurate forecast with a success rate of 92.5% (7.5% error rate in the classification). Of the 40 samples analyzed in the testing dataset only 3 were misclassified.
Conclusions: The proposed approach accurately classify control and PD patients using DNA methylation data and a neural network algorithm. An avenue of future work is to increase the number of patients analyzed and further test the robustness of the model.
INFLUENCE OF THE E4 ALLELE OF APOLIPOPROTEIN (APOE4) ON ASTROCYTIC AND NEURONAL ACETYLATION LANDSCAPES

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Aims: The ApoE4 gene has been established as the strongest genetic risk factor for late-onset Alzheimer's disease (LOAD) and α-Synucleinopathies, being more prominently associated with Dementia with Lewy bodies (DLB) than Parkinson's disease (PD). However, the mechanisms by which ApoE4 contributes to aetipathogenesis of either of these diseases are still unclear. ApoE is the major lipoprotein in the CNS, predominately produced by astrocytes, playing an important role in transport and neuronal uptake of cholesterol. We hypothesized that the presence of its e4 allelic variant could impact cellular epigenetic regulations and hence modify cellular functions. Thus, we investigated the influence ApoE4 holds on neuronal and astrocytic acetylation-related epigenomes.

Methods: The genome-wide epigenomic distribution of two acetylated histone marks (H3K27ac and H3K9ac) was assessed by CUT & TAG followed by deep sequencing, on magnetically isolated neurons and astrocytes (Miltenyi) from the hippocampus of ApoE4 and ApoE3 (control) Knock In mouse models (027894, Jackson Laboratory).

Results: Epigenomic data revealed differential H3K27ac/H3K9ac enrichments in actrocytes and neurons derived from ApoE4 versus ApoE3 KI mice, with upregulation of genes related to ECM processes present in the ApoE4 KI model. Moreover, various genes related to lipid metabolism were also found to be dysregulated in the aforementioned model.

Conclusions: Overall, our findings show differences in cell-type specific epigenetic profiles in a mouse model expressing APOE4 allele, suggesting that astrocyte-derived ApoE4 is capable to influence both astrocytic and neuronal acetylation regulations. We hypothesize that the effect could be due to dysregulations of the lipid metabolism observed in our study.
**Aims:** Prodromal Parkinson’s disease (PD) is often characterized by alpha-synuclein (Asyn) pathology in both the gut and locus coeruleus (LC), leading to gut damage and noradrenergic phenotypes. We generated a novel transgenic mouse that expresses human Asyn in noradrenergic neurons (DBH-hSNCA), namely in the LC. This model allows us to examine the role of Asyn in LC pathology amidst a PD-relevant second hit: experimental colitis. The primary hypothesis of this study is that endogenous Asyn predisposes the LC to injury during gut inflammation.

**Methods:** DBH-hSNCA mice and non-transgenic littermates (8-10 weeks old) were given dextran sulfate sodium (DSS) in 3 bouts of 5d followed by 5d of recovery for a total of 30d or vehicle. Colon and LC pathology were assessed with a battery of histological and biochemical assays. Additionally, to evaluate whether gut inflammation and LC damage coalesce to drive parkinsonian neurodegeneration, nigrostriatal integrity was explored using histological and biochemical assays.

**Results:** Preliminary data suggest the colon of DBH-hSNCA mice is more vulnerable to colitis, and a mechanistic investigation of this vulnerability is underway. Further, LC damage, nigrostriatal injury, and Asyn pathology after colitis will be discussed.

**Conclusions:** This study will elucidate how an inflamed gut drives LC degeneration especially in the presence of Asyn. Using DSS as a model of gut inflammation and the DBH-hSNCA mouse to model early LC injury, we can explore the relationship between prodromal dysfunction in these tissues, which will have considerable implications for the design and timing of PD diagnostic tools and therapies.
Aims: Parkinson’s Disease (PD) is a progressive neurodegenerative disorder. So far, research mostly focused on dopaminergic dysfunction and resulting motor symptoms. However, in the prodromal phase, PD patients also report non-motor symptoms, e.g. gait disturbances. Interestingly, gait alterations are unresponsive to dopaminergic pharmacological treatments, suggesting an involvement of additional neurotransmitter systems. Pink1 mutations are the second most common cause of autosomal recessive PD. Pink1-KO mouse models show gait alterations similar to those observed in human PD patients, while the underlying mechanism remain mostly elusive.

Methods: In a conditional mutagenesis approach, Pink1 was selectively ablated in serotoninergic, dopaminergic and motoneurons and its effect on gait was analysed. Gait of young and aged mice was assessed using the automated Catwalk® system. Hind limb muscle innervation of a Pink1-full-KO and the conditional lines was analysed using electromyography recordings (EMG) of the tibialis anterior and the gastrocnemius (GST) muscles.

Results: Catwalk gait analyses of Pink1-full-KO mice revealed gait impairments resembling those of PD patients. In addition, Pink1-full-KO mice showed an aberrant innervation of the GST in the EMG and atypical foot movements upon stimulation of the peroneal nerve. This specific gait and physiological phenotype of Pink1-full-KO mice could not be reproduced in serotoninergic and only partially in dopaminergic and motoneuron-conditional Pink1-KO lines.

Conclusions: The observed phenotypes of conditional Pink1-KO lines suggest a limited involvement of dopaminergic and motoneurons in the gait phenotype of Pink1-full-KO mice but exclude the serotoninergic system, i.e. via a modulation of the central pattern generator in the spinal cord.
Aims: Accumulating evidence indicate that alterations in the gastrointestinal (GI) function and the gut microbiota represent a risk factor for Parkinson’s disease (PD). Changes in the gut-brain axis can affect both the enteric and central nervous systems, which might have implications in understanding disease pathophysiology and for the development of disease modifying therapeutic strategies.

Methods: To clarify how GI dysfunction is involved in disease pathogenesis and/or in modulating the manifestation of PD symptoms, we characterized the GI function and the key mechanisms involved in the gut-brain axis of a new humanized transgenic PD mouse model (Tg-Th-hTyr) that progressively accumulates neuromelanin in all catecholaminergic nuclei of the brain, including the dorsal motor nucleus of the vagus nerve. We have performed a battery of motor and non-motor behavioral tests to assess the phenotype of these animals, including the GI function. In addition, we have evaluated gut dysbiosis in fecal samples by 16S RNA gene sequencing and metabolomics, intestinal inflammation using cytokine profiling and histological examination of Tg and wild-type (wt) littermates.

Results: show impaired motor activity in Tg mice compared to wt at 6 months of age. We also detected increased fecal output in Tg mice placed in a novel environment, suggesting alterations in the hypothalamic-pituitary-adrenal (HPA) axis. We also observed a significant increase in body weight and water/food intake in Tg mice.

Conclusions: Our results indicate that the gut-brain axis is altered in our PD mice model and that this model can contribute to clarify the role of gut dysfunction in PD pathogenesis.
EFFECT OF GUT MICROBIAL ALTERATIONS ON MOTOR, GASTROINTESTINAL, AND BEHAVIORAL PHENOTYPE IN A SNCA TRANSGENIC MOUSE MODEL OF PARKINSON’S DISEASE

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Aims: We aimed to provide further characterization of a transgenic mouse model of PD that displays motor and gastrointestinal (GI) symptoms, by examining behavioral symptoms and gut microbiota composition over multiple timepoints. Furthermore, we explored if alterations to the gut microbiota impacted the disease phenotype.

Methods: A panel of motor, GI, and behavioral tests were performed at multiple timepoints on an established PD model (transgenic SNCA\textsuperscript{A53T} mice). Results were compared with wild-type mice of a similar genetic background. Fecal microbiota composition of both strains was determined by 16S sequencing. A subset of mice was treated with antibiotics and the effect on PD phenotype observed. A group of transgenic mice were cohoused with wild-type mice to facilitate the transfer of gut bacteria, and the effect on PD phenotype observed.

Results: We found that transgenic mice displayed mild motor deficits, GI dysfunction, and behavioral alterations in comparison to wild-type mice. This PD phenotype became apparent at 6 weeks and remained non-progressive until 22 weeks of age. Transgenic and wild-type mice also displayed distinct gut microbiota communities. Alterations to the gut microbiota, either through antibiotic treatment or transfer of commensal species via cohousing, had a minimal effect on the PD phenotype of transgenic mice.

Conclusions: This mouse model recapitulates understudied non-motor PD symptoms; however, the phenotype appears to be early-onset and non-progressive. Although these mice display evidence of a “dysbiotic” gut microbiota, microbial alterations have a limited effect on the PD phenotype. These mice may represent a model of PD where the microbiota plays a minor role.
EARLY OLFACTORY BULB PATHOLOGY IN ALPHA-SYNUCLEIN TRANSGENIC RATS

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Aims: Induction of the Chaperone Mediated Autophagy (CMA) pathway via upregulation of LAMP2A receptor is associated with enhanced α-synuclein (AS) clearance and amelioration of its pathological effects (Xilouri et al., 2003). It is unknown whether the CMA enhancement may be beneficial in synucleinopathy models in which pathology is already established, foreshadowing potential clinical use. The aim of the current study is to identify the time point at which abnormal AS accumulation commences in the olfactory bulb of WT hAS overexpressing BAC transgenic rats and to investigate whether the AAV-mediated overexpression of LAMP2A will counteract and/or reverse the aberrant AS deposition and its resultant behavioral effects both in early and later stages.

Methods: We demonstrate via fractionated Western blotting the accumulation of total, human and phosphorylated AS in both Triton- and SDS-soluble fractions of the olfactory bulb of 4, 8 and 12 week-old hAS homozygous BAC rats, relative to WT littermates.

Results: The amount of AS remained constant over 4 to 8 weeks, while an increase was observed at 12 weeks. An olfactory discrimination test revealed potential impairment of olfaction at 12 weeks.

Conclusions: Our data suggest that early aberrant AS accumulation appears in the olfactory bulb at the age of 4 weeks, whereas at the age of 12 weeks there is slight enhancement of pathology and initial signs of olfactory dysfunction. These findings set the stage for the assessment of the effects of the manipulation of the CMA pathway on the olfactory system of this synucleinopathy model. Work is supported by GSRT-HFRI grand (HFRI-FM17-3013).
POSTERS

CAN BACTERIA ENDOTOXIN LIPOPOLYSACCHARIDES (LPS) MODULATE THE EFFECT OF ALPHA-SYNUCLEIN PREFORMED FIBRILS IN THE BRAIN?

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Aims: Preformed fibrils of alpha-synuclein (PFFs) have been widely used to mimic the pathological characteristics of Parkinson’s disease, as PFFs can seed and transmit endogenous alpha-synuclein (aSyn) to phosphorylate and accumulate in LB-like structures, but this transmission in WT mice appears to be relatively slow. Our study aims to investigate if systemic inflammation could accelerate the spread of aSyn pathology in C57BL/6J mice.

Methods: PBS/PFFs (dosage: 3 mg PFF) were unilaterally injected into the medial forebrain bundle (MFB), which was then challenged by one single intraperitoneal injection of PBS/LPS (dosage: 1 mg/kg). The behavior was examined 90 days after the injection of PBS/PFF, and immunohistochemical techniques for phosphorylated aSyn and other molecular effects were then examined.

Results: Phosphorylated aSyn was observed in various brain regions with a significant loss of tyrosine hydroxylase immunoreactive positive neurons in the substantia nigra ipsilateral to the injection. PFFs reduced motor functions of mice and hippocampal spatial memory, but LPS did not significantly exacerbate the effect of PFFs.

Conclusions: Our results agree with other reports showing that aSyn PFFs injected into the brain spread across the brain, but non-sterilized systemic inflammation does not accelerate the spreading process.
AGE-DEPENDENT VARIABILITY IN ALPHA-SYNUCLEIN INDUCED DOPAMINERGIC NEURODEGENERATION IN MICE AFTER LOCAL EXPOSURE OF ROTENONE IN OLFACTORY BULBS

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Aims: Parkinson’s disease is an age-related neurodegenerative disorder. Aim of the present study was to check the age dependent effect of intranasal rotenone microemulsion (ME) on alpha-synuclein (α-syn) accumulation induced dopaminergic (DAergic) neurodegeneration in olfactory bulb (OB) and mid brain.

Methods: We administered rotenone ME intranasally at a dose of 0.1mg/kg (which is not detectable in blood and brain) in 3 months (young) old mice for 9 weeks followed by 16 weeks of wash-out period. However, one year old (aged) mice received rotenone for 6 weeks only. Behavioral studies for olfactory and motor impairment were performed. Further, the mice were sacrificed and brain samples were analyzed for α-syn accumulation, neuroinflammation and neurodegeneration in the OB, striatum and substantia nigra pars compacta (SNc).

Results: Behavioral studies with young and aged mice showed olfactory and motor impairment along with increase in α-syn and phosphorylated α-syn levels in the OB and striatum. However, young mice did not show glial cell activation and dopaminergic neuronal loss, while aged mice were able to show both glial cell activation and loss of dopaminergic neurons in OB, striatum and SNc. To further check the aging effect, we performed the iron staining in aged mice and found it to be significantly increased in SNc in rotenone-treated mice as compared to control.

Conclusions: Our studies suggest the difference in susceptibility of dopaminergic neurons towards aging factors as indicated by alpha-synuclein accumulation induced degeneration of dopaminergic neurons in aged mice.
Aims: Parkinson’s disease (PD) is a basal ganglia movement disorder characterized by progressive degeneration of the nigrostriatal dopaminergic system. Immunohistochemical methods have been widely used for characterization of dopaminergic neuronal injury in animal models of PD, including the MPTP mouse model. However, conventional 2D immunohistochemical techniques have inherent limitations with respect to spatial resolution, yielding insufficient information on the architecture of the dopaminergic system. Here, we aimed to provide a comprehensive and non-biased 3D map of MPTP-induced central dopaminergic pathway changes with light sheet fluorescence microscopy (LSFM) and deep-learning computational methods.

Methods: Mice terminated seven days after acute MPTP administration (four successive injections of 20 mg/kg, IP, once every 2h). The brains were stained and cleared with iDISCO protocol and scanned with LSFM. A deep-learning computational method was applied to quantify tyrosine hydroxylase (TH) positive neurons in the brains.

Results: Compared to vehicle controls, MPTP mice showed a significant loss of TH-positive neurons in the substantia nigra pars compacta and ventral tegmental area. Also, MPTP treatment reduced overall TH signal intensity in basal ganglia nuclei, i.e. the substantia nigra, caudate-putamen, globus pallidus and subthalamic nucleus. In contrast, increased TH signal intensity was predominantly observed in limbic regions, including several subdivisions of the amygdala and hypothalamus.

Conclusions: Mouse whole-brain 3D imaging is ideal for unbiased counting and densitometric analysis of TH-positive cells. The LSFM-deep learning pipeline tracked brain-wide changes in catecholaminergic pathways in the MPTP mouse model of PD and may be applied for preclinical characterization of compounds targeting dopaminergic neurotransmission.
Aims: Degeneration of nigrostriatal dopaminergic neurons in PD begins from the axons in the striatum before the onset of motor symptoms. This study aimed to develop a progressive neurodegeneration process of the dopaminergic axons for further testing potential neuroprotectors.

Methods: Mice received 4 injections of 12 mg/kg 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) each 2h. Then, from 6h up to 24h after 1st MPTP injection, we estimated in the striatum: the number of tyrosine hydroxylase (TH) axons (IHC), the concentration of dopamine (HPLC) and content of TH (WB), TH activity as L-DOPA accumulation upon inhibition of aromatic L-amino acid decarboxylase (HPLC). Also, nomifensine (10 mg/kg), the dopamine transporter inhibitor, was administered 30min before each MPTP injections and the dopamine level and TH-axons were detected 12h later.

Results: Degradation of axons in the striatum began immediately after MPTP administration and lasted for 12h, which was accompanied by acute decrease in dopamine concentration and low TH activity. Subsequently, the number of axons and dopamine content did not change. From 12h up to 18h TH content was decreased, and its activity was increased that due to stabilization of the dopamine level. This model was validated using neuroprotector with well-known mechanisms of action: nomifensine, which was shown to almost protect dopaminergic axons from the MPTP toxic effect and maintain the striatal dopamine concentration at the control level.

Conclusions: The developed model reproduced the key element of early PD pathogenesis: gradual dopaminergic axons degeneration, and it can be used for testing of the neuroprotectors. This study was supported by RSF (project № 20-75-00110).
A SUBCHRONIC MODEL OF PARKINSON'S DISEASE IN MICE THAT REPRODUCES THE PROGRESSIVE DEVELOPMENT OF THE DISEASE

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Aims: Low efficiency of Parkinson's disease (PD) treatment is due to late diagnosis under degradation of most nigrostriatal dopaminergic neurons (DN). Curing PD can be improved if developing early diagnosis and preventive treatment, which is possible only in models of PD progressing development. Development of such a model is the goal of this study.

Methods: Mice C57Bl/6 were received 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) injections for seven days in an increasing dose of 8, 10, 12, 16, 20, 26, 34, 46 mg/kg. The concentration of striatal dopamine and metabolites (HPLC), the number of DN (IHC) and the content of dopamine in the substantia nigra were daily assessed. After the 5th day motor behavior (Open field) was evaluated.

Results: As the MPTP dose increased from 8 to 12 mg/kg, the striatal dopamine concentration gradually decreased to 30%, remaining at this level up to MPTP dose 34 mg/kg. At a dose 46 mg/kg, the dopamine concentrations dropped below the threshold (30%) and motor disorders were detected. Dopamine turnover gradually compensatory increased until MPTP was administered at a dose 26 mg/kg. However, at a dose 34 mg/kg, dopamine turnover returned to control, due to decompensation. The number of DN and dopamine content in the substantia nigra began to decrease after MPTP dose 16 mg/kg.

Conclusions: Development of a subchronic model of progressive degradation of the nigrostriatal system in PD in this study gives new opportunities for developing early diagnosis and treatment. Study was funded by Ministry of Science and Higher Education of the RF (grant agreement № 075-15-2020-795 of 29.09.2020).
NEUROMELANIN-LIKE PIGMENTATION IN MOUSE LOCUS COERULEUS LEADS TO SEVERE DYSFUNCTION, DEGENERATION, AND IMMUNE RESPONSE

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Aims: Neuromelanin (NM) is uniquely expressed in dopamine neurons of the substantia nigra (SN) and norepinephrine neurons of the locus coeruleus (LC), two regions selectively vulnerable in Parkinson’s disease (PD). NM is a cytosolic pigment comprised of granules containing a combination of catecholamine metabolites, lipid droplets, protein aggregates and heavy metals. NM serves a neuroprotective role as it accumulates across the lifetime, but may become harmful when it is released from these cells as they degenerate in PD. To study the effects of NM on the non-motor symptoms of PD, we have adapted a rodent model expressing NM-like pigmentation in the LC.

Methods: Tyrosine hydroxylase-cre (TH-cre) mice (n = 10) and littermate controls (n = 10) were injected with a cre-dependent AAV5-human Tyrosinase (hTyr), leading to NM-like pigmentation subsequent neurodegeneration. Mice were assessed on a battery of non-motor behavioral tests at 2, 5, or 10-weeks following injection. Cell integrity, immune response, and protein aggregation was then measured through immunohistochemistry.

Results: Pigment-expressing mice showed severe LC dysfunction and degeneration by 10-weeks compared with controls. This cell death was coupled with a robust immune response and altered non-motor behavioral phenotypes. NM-like granules were retained despite cell body loss, indicating extracellular expression.

Conclusions: Expression of NM-like pigmentation in mice leads to disrupted behavioral phenotypes and degeneration of the LC, indicating potentially neurotoxic mechanisms of NM in PD.
DEMENTIA-LINKED TDP-43 DYSREGULATION IN ASTROCYTES IMPAIRS MEMORY, ANTIVIRAL SIGNALING, AND CHEMOKINE-MEDIATED ASTROCYTIC-NEURONAL INTERACTIONS

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Aims: TDP-43 pathology is linked to cognitive deficits in diverse neurodegenerative disorders, including frontotemporal dementia (FTD) and Alzheimer’s disease (AD). This study investigated whether astrocytic TDP-43 is dysregulated in human brain and how this dysregulation affects behavior, gene expression, and neural activities.

Methods: We used postmortem human brain samples, extensive behavioral testing in different cohorts of transgenic mice, gene profiling, synaptic analyses, glial-neuronal co-culture assays and physiology, and biochemical assays to identify signaling cascades linked to TDP-43.

Results: Our results show that astrocytic TDP-43 is mislocalized in postmortem human hippocampal tissue from AD cases. To assess the effects of widespread or hippocampus-specific dysregulation of astrocytic TDP-43 in complementary systems, we generated three astrocyte-specific mouse models of TDP-43 dysfunction. Consistently, these mouse models had progressive hippocampus-dependent memory loss. Manipulation of astrocytic TDP-43 also increased expression of astrocytic antiviral genes and interferon-inducible chemokines CXCL9 and CXCL10, and impaired astrocytic defense against viral pathogens. Moreover, expression of CXCR3, the shared receptor for CXCL9 and CXCL10, was increased selectively in presynaptic terminals, and stimulation of CXCR3 modulated neuronal activities and presynaptic vesicles.

Conclusions: Our findings shed new light on TDP-43 dysregulation in astrocytes and its contributions to impairments in cognitive and immune-related functions. We report a novel chemokine-mediated astrocytic-neuronal pathway that is likely downstream of aberrant antiviral immune signaling in astrocytes that affects presynaptic release and neuronal activities. Together, these results implicate cell-autonomous astrocytic TDP-43 dysregulation in the pathogenesis of dementia and point to astrocytic chemokine signaling and neuronal CXCR3 as potential therapeutic targets for alleviating cognitive decline.
A CASE–CONTROL SEROPREVALENCE STUDY ON THE ASSOCIATION BETWEEN TOXOCARIASIS INFECTION AND PARKINSON AND ALZHEIMER DISEASES

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Aims: Toxocariasis is a zoonotic disease that human infection occurs by eating the embryonated eggs of Toxocara spp. Various studies have investigated the association of toxocariasis with central nervous system (CNS) disorders and in some cases, a significant association has been found. The aim of the present study was to examine the association between toxocariasis infection and Parkinson’s disease (PD) and Alzheimer’s diseases (AD).

Methods: A total of 186 patients with AD and PD (93 patients in each group) and as the control group, 93 healthy people that were statistically matched with the case group were included in the study. Blood samples were collected from all participants and the anti-Toxocara IgG antibodies were detected using the ELISA kit.

Results: The overall seroprevalence in all participants was 5.1% (95% CI, 4.5-5.6%; 14/279). Anti-Toxocara IgG antibodies were found in 8/93 PD cases (8.6%; 95% CI, 7-10.1%), 3/93 AD cases (3.2%; 95% CI, 2.6-3.7%), and in 3/93 healthy controls (3.2%; 95% CI, 2.6-3.7%) (Fig. 1). There were no significant associations regarding the Toxocara infection seropositivity and PD (OR, 2.82; 95% CI, 0.72-11.00) and AD (OR, 1.00; 95% CI, 0.20-5.09). Moreover, considering potential risk factors for Toxocara infection seropositivity, we have not found any significant variable in both univariate and multivariate analyses.

Conclusions: There was no significant association regarding the Toxocara infection seropositivity and PD (OR, 2.82; 95% CI, 0.72-11.00) and AD (OR, 1.00; 95% CI, 0.20-5.09). Based on the results, it could be concluded that there was no significant relationship between AD, PD, and Toxocara infection.
POSTERS

LOSS OF TREM2 REDUCES HYPERACTIVATION OF PROGRANULIN DEFICIENT MICROGLIA BUT NOT LYROSOMAL PATHOLOGY


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Aims: GRN haploinsufficiency causes frontotemporal lobar degeneration and results in microglial hyperactivation, lysosomal dysfunction and TDP-43 deposition. To understand the contribution of microglial hyperactivation to pathology we evaluated genetic and pharmacological approaches suppressing TREM2 dependent transition of microglia from a homeostatic to a disease associated state.

Methods: We created GRN deficient human iPSC lines and differentiated them into human iMicroglia. We characterized them and found they were hyperactivated and had pathologically increased lysosomal function. To explore antibody-mediated pharmacological modulation of TREM2-dependent microglial states, we identified antagonistic TREM2 antibodies.

Results: Treatment of PGRN deficient hiMGL showed dampened microglial hyperactivation, reduced TREM2 signaling and phagocytic activity, however, lysosomal dysfunction was not rescued. Furthermore, data from GRN KO mice indicated that genetic loss of TREM2 even elevated NfL, a biomarker for neurodegeneration.

Conclusions: These findings suggest that microglia hyperactivation is not necessarily contributing to neurotoxicity, and instead demonstrates that TREM2 exhibits neuroprotective potential.1

Aims: Proteinaceous inclusions of TDP-43 are hallmark pathological features in most cases with amyotrophic lateral sclerosis (ALS), ~50% of cases with frontotemporal dementia (FTD) and >60% of cases with Alzheimer’s disease (AD). TDP-43 regulates many facets of RNA metabolism; thus, TDP-43 loss-of-function in disease results into the accumulation of key aberrant transcripts. One of these transcripts may be the recently discovered variant of a protein involved in axonal regeneration: stathmin-2 (STMN2). The accumulation of a truncated variant of STMN2 (tSTMN2), with a concurrent decrease in the full-length STMN2 transcript has been shown in ALS, resulting from TDP-43 loss-of-function.

Methods: Postmortem brain tissues were evaluated for STMN2 RNA levels, as well as their association with phosphorylated TDP-43 (pTDP-43) protein burden and clinical features. Finally, levels of full-length STMN2 protein were also assessed and studies are ongoing to determine the contributions of the loss of STMN2 function to disease, as well as the biomarker potential for STMN2 variants in TDP-43 proteinopathies.

Results: In FTLD-TDP, tSTMN2 is significantly elevated in frontal cortex where it correlates with TDP-43 burden, and with age at onset. Our exciting preliminary data demonstrates STMN2 missplicing in the hippocampus of AD cases with TDP-43 pathology.

Conclusions: STMN2 missplicing occurs in AD tissues affected by TDP-43 pathology. Ongoing studies are aimed to evaluate the consequences of STMN2 missplicing in disease, determine if particular TDP-43 subtypes are more susceptible to these splicing changes, identify misspliced TDP-43 targets unique to AD, and evaluate biomarker potential of missspliced STMN2 variants for AD and other TDP-43 proteinopathies.
C9ORF72 ASSOCIATED DIPEPTIDE PROTEIN REPEAT OLIGOMERS IN ALS AND FTD

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Aims: The hexanucleotide repeat expansion in C9orf72 is one of the most common causes of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). The hexanucleotide expansion generates toxic dipeptide protein repeats including GP, GA, GR, PA, and PR. Increasing evidence suggests that soluble species, like oligomers, are the main cause of neurotoxicity in neurodegenerative conditions such as Alzheimer’s and Parkinson’s disease however, this is unclear in ALS and FTD. Here, we investigated the ability of DPRs to aggregate and form toxic oligomers as well as interact with amyloidogenic proteins like tau.

Methods: We have taken a reductionist approach by synthesizing short dipeptides repeats (GA, PR, and GR) of varying lengths and used biophysical as well as biochemical assays to characterize the aggregates in vitro and in cellular models. We also used immunoblotting and immunostaining to detect DPR oligomers in ALS and FTD patients.

Results: The results suggest the propensity for DPRs, especially GR and PR, to form oligomeric structures which can cause toxicity. We also tested the toxicity of the varying DPR aggregates alone and in the presence with tau protein in cellular models. Moreover, we investigated the presence of DPR oligomers in ALS and FTD human samples and their potential role in disease pathogenesis.

Conclusions: Many studies have investigated the toxicity associated with DPRs however it is still unclear the role of DPRs oligomers in disease progression. Thus, the ability to detect and characterize oligomeric DPRs has great potential to further the understanding of these diseases and aid in the development of targeted therapeutics.
TDP-43 PATHOLOGY AND PRIONIC BEHAVIOUR IN HUMAN CELLULAR MODELS OF ALZHEIMER’S DISEASE PATIENTS

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Aims: Alzheimer's disease (AD) is a neurodegenerative disorder for which there is currently no effective treatment. Despite advances in the molecular pathology of the characteristic histopathological markers of the disease (tau protein and β-amyloid), their translation to the clinic has not provided the expected results. Recent studies have reported the relevance of TDP-43 (TAR DNA binding protein 43) in AD. Increasing evidences have demonstrated the presence of these aggregates in the postmortem brains of patients diagnosed with AD. The present research is focused on the study of the pathological role of TDP-43 in AD.

Methods: For this purpose, immortalized lymphocytes samples from patients diagnosed with different severity of sporadic AD were used and the TDP-43 pathology was analyzed against controls, looking for differences in their fragmentation, phosphorylation and cellular location using Western blot and immunocytochemical techniques. Moreover, extracellular media from these cultures have been also analyzed.

Results: The results revealed an increase in TDP-43 fragmentation, as well as increased phosphorylation and aberrant localization of TDP-43 in the cytosolic compartment of lymphocytes of patients diagnosed with severe AD. Moreover, a fragment of approximately 25 KD was found in extracellular vesicles (EVs), isolated from conditioned medium of cells derived from severe AD individuals, that seems to have prion-like characteristics.

Conclusions: TDP-43 plays a key role in AD pathogenesis and in its cell to cell propagation. More studies are needed to decipher the exact role of this nuclear protein in AD and to translate these data to future therapeutics.
Aims: The abnormal aggregation of transactive response DNA-binding protein of 43 kDa (TDP-43) in neurons and glia is the defining pathological hallmark of amyotrophic lateral sclerosis (ALS) and multiple forms of frontotemporal lobar degeneration (FTLD). It is also common in other diseases, including Alzheimer's and Parkinson's. However, the structures of pathological aggregated TDP-43 are unknown. 

Methods: We used electron cryo-microscopy (cryo-EM) to determine the structures of aggregated TDP-43 extracted from the frontal and motor cortices of individuals that succumbed to ALS with FTLD.

Results: We found a conserved amyloid-like filament structure comprising a single protofilament. The ordered filament core is formed by residues 282 to 360 in the TDP-43 low-complexity domain, which adopt a novel double-spiral-shaped fold. The fold shows no similarity to those of TDP-43 filaments formed in vitro. Abundant glycine and neutral polar residues facilitate numerous turns that restrict b-strand length, resulting in the absence of b-sheet stacking associated with cross-b amyloid structure. An uneven distribution of these residues gives rise to structurally and chemically distinct filament surfaces. External densities adjacent to these surfaces suggest possible ligand binding sites.

Conclusions: This work enhances our understanding of the molecular pathogenesis of ALS and FTLD, revealing the formation of filaments that are structurally distinct from amyloid filaments in other neurodegenerative diseases. The structure of pathological TDP-43 filaments in ALS-FTLD informs the development of accurate disease models, as well as diagnostic and therapeutic agents.
SUBCOMMISSURAL ORGAN-SPONDIN-DERIVED PEPTIDE IMPROVES MOTOR FUNCTION AND PROLONGS SURVIVAL IN A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Aims: NX210 is an innovative drug candidate peptide with pleiotropic functions derived from the subcommissural organ (SCO)-spondin, a large glycoprotein that strongly contributes to neuronal development. The aim of this study was to evaluate the therapeutic effect of the linear and cyclic (NX210c) forms of the peptide in the SOD1⁴ G93A mouse model of ALS.

Methods: Female SOD1⁴ G93A mice were treated with vehicle or different doses of NX210 and NX210c (2.5, 5 or 10 mg/kg) from 90 days old until disease end-stage (n=12-15/group). The static rods test was performed every other week to evaluate motor deficits, and the clinical score was evaluated twice a week to determine survival.

Results: Treatment with NX210 and NX210c at 10 mg/kg significantly reduced ALS-induced increased orientation and travel times from 16 weeks old (WT: 1.3s and 3.2s, vehicle SOD1⁴ G93A: 24.5s and 26.6s, NX210 SOD1⁴ G93A: 2.8s and 5.4s, NX210c SOD1⁴ G93A: 2.6s and 4.8s for orientation and travel times, respectively) until disease end-stage in the static rods test. Interestingly, both peptide forms at 10 mg/kg extended the lifespan of SOD1⁴ G93A mice; the median survival of vehicle-treated SOD1⁴ G93A mice was 143 days whereas treatment with NX210 and NX210c increased the lifespan of SOD1⁴ G93A mice to 148 and 153.5 days, respectively.

Conclusions: NX210c represents a promising disease-modifying drug candidate for ALS. Indeed, it prolongs mouse survival and reduces motor deficits in the SOD1⁴ G93A mouse model. Spinal cords were collected at 16 weeks old to investigate the cellular mechanisms underlying the beneficial effects of NX210c on ALS disease progression/survival.
THE EXTRACELLULAR PROGRANULIN/PROSAPOSIN COMPLEX MEDIATES LYSOSOMAL SIGNALING AND TARGETS MICROGLIA AND NEURONS IN VIVO

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Aims: GRN-related frontotemporal dementia (FTD/GRN) is caused by progranulin haploinsufficiency and is characterized by aberrant microglial activity, neuroinflammation and TDP pathology-associated neurodegeneration. Progranulin is expressed in neurons and microglia and plays a pivotal role for lysosomal function, including a regulatory role of glucocerebrosidase (GCase) activity. Progranulin is a secreted protein recently suggested to interact with prosaposin, another lysosomal protein essential for GCase activity. In this project we have studied the progranulin/prosaposin complex and asked what cell types it targets in the brain and whether it regulates GCase activity in cell culture studies.

Methods: Progranulin/Prosaposin complexes were purified from conditioned media of cells co-expressing progranulin and prosaposin. Purified progranulin/prosaposin complexes were added to human fibroblast cultures, mouse primary neuronal cultures or administered intracerebroventricularly in rats using Alzet mini pumps. Brains and cells were subsequently analyzed with immunohistochemistry and biochemistry for cellular targeting and glucocerebrosidase activity, respectively.

Results: Progranulin/Prosaposin complexes showed a stronger binding to primary neurons as compared to progranulin or prosaposin alone. Furthermore, the progranulin/prosaposin complex demonstrated an efficient lysosomal targeting and colocalization with GCase in human fibroblasts and stimulated GCase activity in both cell types. Intracerebroventricularly administered progranulin/prosaposin diffused into the brain and targeted both microglia and neurons.

Conclusions: Our data show that extracellular progranulin/prosaposin complexes interact with key cell types affected in the FTD/GRN brain and stimulate GCase activity, a pivotal downstream target for progranulin and prosaposin signaling. These studies provide a framework to study progranulin/prosaposin in disease and as a potential therapeutic for FTD/GRN.
NEUROPATHOLOGICAL, CLINICAL AND GENETIC COMPARISON OF 3 PECULIAR FTLD CASES FROM THE ABBIATEGRASSO BRAIN BANK

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Aims: Objectives The spectrum of fronto-temporal lobar degeneration (FTLD) encompasses several proteinopathies and clinico-pathological features. The aim of the study is to understand if in FTLD, behavioral and non-behavioral symptoms depend on the topography of the lesions or on the type of protein aggregates and their interaction.

Methods: Methods Three subjects with major-neurocognitive disorder (major-NCD), belonging to the Abbiategrasso Brain Bank, underwent serial neurological and neuropsychological evaluations. Their brains were processed to evaluate the vascular and degenerative lesions (see protocol Jove 2020).

Results: Case1 (major frontotemporal NCD: nfPPA/CBS): progressive non-fluent aphasia (nfPPA), apraxia, severe right hemiparkinsonism, executive deficit, disinhibition, apathy and hyperorality; neuropathology: prevalent type A TDP-43 histopathology but with limbic co-localization of synuclein pathology. Case2 (major frontotemporal NCD: bvFTD): progressive postural instability, bilateral parkinsonism, spatial disorientation with left visual perception deficit, wandering, disinhibition, delusions, apathy and hyperorality; neuropathology: pure type A TDP-43 histopathology. Case3 (major-NCD due to AD: behavioral AD) progressive memory loss, logopenic language impairment, social withdrawal, apathy, aggressive behavior, wandering, sugar craving, sleep disturbances, delusions and hallucinations; neuropathology: predominant AD pathology, limbic synuclein pathology and, to a lesser extent, TDP-43 pathology.

Conclusions: Conclusion The clinico-pathologic comparison of these cases demonstrates protein co-localization in FTLD confirming a possible pathological synergic role of TDP-43, synuclein and AD pathology, particularly in the limbic system. Indeed, cases with multiple limbic pathologies (1 and 3) show hippocampal sclerosis. TDP-43 cases (1 and 2) have prominent hemispheric asymmetry influencing the clinical phenotype, which depends on the topography of the lesions rather than on their molecular nature.
TDP-43 AS STRUCTURE-BASED BIOMARKER IN AMYOTROPHIC LATERAL SCLEROSIS: FINDINGS FROM A MULTI-CENTER PILOT STUDY

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Aims: Pathologic alterations of Transactivation response DNA-binding protein 43 kilo Dalton (TDP-43) are a major hallmark of amyotrophic lateral sclerosis (ALS). One of the key challenges in ALS diagnostics is to exclude other mimicking diseases, while disease-specific diagnostic and prognostic biomarkers are still lacking. In this pilot study we therefore analyzed the potential of the secondary structure distribution of TDP-43 in CSF of ALS patients, Parkinson’s disease (PD) patients and further controls to be a disease specific biomarker candidate for ALS.

Methods: Lumbar punctures and neuropsychological assessments were performed at specialized clinics. An immuno-infrared-sensor was used for the detection of the biomarker secondary structure distribution, which directly reflects protein misfolding, in CSF of ALS patients (n=36) compared to PD (n=30) and further controls (n=24). The whole TDP-43 fraction was extracted by capture antibodies on a chemical functionalized surface. Subsequently the secondary structure distribution was examined via infrared difference spectroscopy and correlated with the ALS functional rating scale – revised score (ALSFRS-R).

Results: The secondary structure distribution analyses revealed, that ALS patients had significantly more misfolded TDP-43 compared to PD and controls (P < 0.0001). ALS patients could be discriminated from PD and controls with a sensitivity-specificity of 89 %/77 % and 89 %/83 %, respectively. Moreover, misfolding of TDP-43 was positively correlated with the ALSFRS-R scores (P = 0.03, r = 0.37).

Conclusions: In conclusion, we present for the first time a TDP-43 structure-based biomarker for ALS, which should be validated in larger studies.
DIFFERENTIAL GENE EXPRESSION IN TWO KIND OF SAMPLES OF FRONTOTEMPORAL DEMENTIA: CEREBRAL CORTEX AND LYMPHOBLASTOID CELL LINE.

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Aims: To analyze the whole genome differential gene expression in frontotemporal dementia (FTD) samples: prefrontal cortex and lymphoblastoid cell lines (LCLs).

Methods: We studied a total of 41 samples: 28 from brain tissue and 13 LCLs, of the following groups: sporadic FTD-Tau, sporadic FTD-TDP43, MAPT-mutation carriers (MC), C9orf72-MC, GRN-MC and healthy controls. Clariom D microarray (Affymetrix) was used to analyze gene expression. Differentially expressed genes (DEG) were obtained for group pairwise comparisons (pval<0.05, FC>1.5 or FC<1/1.5) and for each tissue independently. Biological significance was analyzed by Gene Set Enrichment Analysis using Reactome and GO databases (10 top pathways).

Results: Most DEG in patients versus controls comparisons were overexpressed in both tissues, except for GRN-MC versus controls comparison in which DEG were mostly down-regulated. Brain and LCLS showed few common DEG. Biological significance analyses in brain tissue revealed metabolism, immune system, synaptic transmission, signaling and response to stimulus as pathways shared by comparisons of both genetic and sporadic FTD patients with respect to controls. In MAPT-MC and GRN-MC versus controls metabolism pathways, but not neuronal pathways, were altered both in brain and LCLS.

Conclusions: Common altered pathways are found in both sporadic and genetic groups of FTD patients compared to controls in brain tissue. Brain and LCLS transcriptomes showed metabolism related pathways dysregulated in genetic FTD groups versus controls.
EDARAVONE REDUCES TDP-43 LEVEL BUT NOT STRESS GRANULE FORMATION IN SODIUM ARSENITE TREATED TDP-43 OVEREXPRESSING CELLS

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Aims: The RNA-binding protein TDP-43 is linked to neurodegenerative diseases like amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). Studies have shown that cytoplasmic TDP-43 aggregates co-localize with stress granule (SG) markers. SGs are cytoplasmic inclusions that repress translation of a subset of RNAs during cellular stress. Since it was shown that SG formation contributes to accumulation of TDP-43, inhibition of SG formation and/or recruitment of TDP-43 to SGs are pathways that are currently in the focus of ALS research. To be able to support this research, we have set up a respective in vitro model in TDP-43-overexpressing neuroblastoma cells.

Methods: Human TDP-43 overexpressing neuroblastoma cells were stimulated with the well-described SG inducer sodium arsenite (SA) and treated with edaravone or cycloheximide as possible rescuing agents. SG formation and TDP-43 recruitment were investigated via immunocytochemistry. TDP-43 aggregation in soluble and insoluble protein fractions was analyzed by ProteinSimple WES technology.

Results: Immunocytochemical staining for the SG marker G3BP revealed substantial SG formation in SA-treated cells compared to the respective vehicle control. Cycloheximide counteracts SG formation, whereas edaravone had no impact on stress granule formation, but on TDP-43 immunoreactive area. WES analysis showed a strong shift from soluble to insoluble TDP-43 species upon SA stimulation which could be attenuated with edaravone treatment.

Conclusions: The presented in vitro model using SA as stressor to induce SGs is a suitable system to study SG formation and TDP-43 aggregation, which are differently affected by the tested agents edaravone and cycloheximide.
TDP43/FUS FLUORESCENCE-BASED ASSAY DEVELOPMENT TO SCREEN DRUGS AGAINST AMYOTROPHIC LATERAL SCLEROSIS DISEASE

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Aims: A novel ALS fluorescence cell-based assay has been designed for High Content Screening applications to find compounds able to inhibit or modulate TDP43/FUS proteins aggregation.

Methods: Cultured cells: The human U2OS cell line has been used to generate a FUS/TDP43 double stable cell line. This cell line has been subcultured in 96 wells Imaging Plates (BD Biosciences) at 0.15 cells/cm² in 200 µl of DMEM F12 10% FBS and incubated at 37 ºC and 5 % CO2. Image acquisition: The cell line expressing human TDP43 protein inducibly and FUS protein constitutively was treated with sodium arsenite during 90 min. Afterwards, the presence of TDP43 and FUS aggregates was quantified by fluorescence using image analysis algorithms.

Results: FUS-FP602 and TDP-43-tGFP chimeras produce enough intensity of fluorescence to be analysed in HCS device. The fluorescence tag doesn’t change the FUS/TDP43 capability to form stress granules under oxidative stress conditions. TDP43 and FUS signaling pathways have been analysed after severe citotoxic damage induction by sodium arsenite, using Riluzole and Arimoclomol as control compounds. This ALS cellular model has been adapted to HCS analysis based on image algorithms to test protein aggregation process.

Conclusions: U2OS TDP43/FUS cell line can be used in drug discovery to search for pathological protein aggregation inhibitors or modulators in HCS screening. This ALS cellular model allows evaluating the TDP-43 and FUS proteins distribution in living cells studying the temporal and spatial protein localization pattern. It may be useful to study the relationship between both proteins during the aggregation process under citotoxic conditions.
A COMMON MICROVASCULAR ENDOPHENOTYPE IN HEAD INJURIES AND ALZHEIMER’S DISEASE

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Aims: Cerebrovascular injury is a common pathological feature of a spectrum of neurological disorders including traumatic brain injury (TBI), stroke, Alzheimer’s disease (AD), as well as aging. Vascular manifestations among these conditions are similar indeed, including the breakdown of the blood-brain barrier (BBB). However, whether there is a unique molecular mechanism underlying the vascular changes among these conditions remains elusive.

Methods: scRNA-seq data processing We re-clustered the subcategorized vascular cell types of mouse mild TBI and aging scRNA-seq datasets and performed secondary analysis with the Seurat Package (v.3.1.5) in R (v.3.6.1). Generation of the Tmem252 Knockout model The murine Tmem252 gene only contains 2 exons, therefore CRISPR/cas9 technology was chosen with gRNAs specifically targeting the 2 exons, resulting in null allele. Closed-head mTBI mouse model After anesthesia, mice received a mild closed-head impact using a 4mm plastic flat-tip impactor, with 3m/s velocity and 1.0 mm depth.

Results: Based on transcriptomic analysis on cerebrovascular single-cell RNA-seq datasets, we identified a common molecular signature between mTBI and aging vasculature, involving a novel transmembrane protein Tmem252. Therefore, to further explore Tmem252-dependent microvascular endophenotype in mTBI and AD, we generated the Tmem252 KO mice and performed mTBI. We found that Tmem252 KO mice are protected from mTBI-induced microvascular injury when compared with their wild-type littermates.

Conclusions: Based on our preliminary data from comprehensive bioinformatic analysis and validations in multiple models of neurological disorders, we propose specific Tmem252 upregulation in BBB is a common molecular signature for microvascular injury.
Aims: To investigate the association of clinical bleeding risk with hemorrhagic features of cerebral amyloid angiopathy (CAA) in neuropathology.

Methods: Retrospective analysis of consecutive autopsies of hospitalized patients in the Department of Rehabilitation and Geriatrics from the University Hospitals of Geneva, performed from 2014 to 2019. Clinical data were collected from medical records, blinded to the autopsy results. The presence or absence of CAA was determined on anti-Aβ-antibody-stained sections and categorized on a four-level grading system of severity. Logistic regression models were used to study the association of CAA with bleeding risk determined by the HAS-BLED score (0-9 points; low risk: <3; high risk: ≥3).

Results: 148 autopsies were performed and the population was composed of 41.2% of male patients, mean age of 83.9 ±7.1 y. A clinical diagnosis of dementia was documented in 33.1% of cases, the most common etiology being Alzheimer's Disease (17 cases; 11.5%). High bleeding risk was present in 64.2% of cases (HAS-BLED ≥3). Patients with HAS-BLED ≥3 had a higher proportion of CAA in temporal (11.3% vs.28.4%; p=0.022) areas. In the regression models, patients with HAS-BLED ≥3 had more than three times and more than two times higher risk of CAA in temporal (OR 3.11, 1.19-8.12 95% CI; p=0.020) and occipital (OR 2.07, 1.02-4.19 95% CI; p=0.042) topographies, respectively. The association with temporal CAA remained significant after adjustments for age and sex (OR 3.03, 1.15-7.97 95% CI; p=0.024).

Conclusions: High bleeding risk is associated with the presence of neuropathological features of CAA in temporal lobes of older patients.
Aims: To investigate the association of clinical thromboembolic risk with the presence of cortical microinfarcts (CMI) in neuropathology.

Methods: Retrospective analysis of consecutive autopsies of hospitalized patients in the Department of Rehabilitation and Geriatrics from the University Hospitals of Geneva, performed from 2014 to 2019. Clinical data were collected from medical records, blinded to the autopsy results. Tissues blocks were systematically taken from different cortical areas and examined with Globus silver staining for optimal visualization of CMI. To determine the cutpoint of the CHA₂DS₂-VASc score best associated with CMI we performed a ROC curve analysis with sensitivity and specificity values. Then, we performed logistic regression models to study the abovementioned association.

Results: 148 autopsies were performed and the population was composed of 41.2% of male patients, mean age of 83.9 ±7.1 y. A clinical diagnosis of dementia was documented in 33.1% of cases, the most common etiology being Alzheimer's Disease (17 cases; 11.5%). At least one CMI was detected in 40.5% of cases and in 14.2% they were present in multiple topographies. Patients with CMI were more often diabetic (31.7% vs. 14.8%; p=0.024) with a higher CHA₂DS₂-VASc score. CHA₂DS₂-VASc ≥5 was the cutpoint for optimal sensitivity and specificity to the association with CMI, with an area under the ROC curve of 0.602. When applying in logistic regression models, a CHA₂DS₂-VASc ≥5 increased by more than two times the risk of presenting CMI in neuropathology (OR 2.23, 1.13-4.39 95% CI; p=0.020).

Conclusions: High thromboembolic risk is associated with CMI in the neuropathology of older patients.
POSTERS

CCR5 DEFICIENCY: DECREASED NEURONAL RESILIENCE TO OXIDATIVE STRESS AND INCREASED RISK OF VASCULAR DEMENTIA.

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Aims: Oxidative stress is thought to contribute to the development of cognitive decline. Chemokine receptor 5 (CCR5) deficient mice show increased stroke size in response to ischemia. Here, we investigate whether there is the link between CCR5 deficiency, the sensitivity of neurons to oxidative stress, and the development of dementia.

Methods: Logistic regression models with CCR5 and ApoE polymorphisms were used as independent variables to assess the genetic risk for different types of dementia in geriatric inpatients. The impact of oxidative stress on mechanisms of CCR5 expression and cell death was assessed in neurons from wild-type and CCR5⁻/⁻ mice.

Results: 394 patients were included in Geneva (205 cognitively normal and 189 demented). Although CCR5-delta32 allele was not an independent predictor of dementia, it synergized with ApoEpsilon4 as risk factor. Subgroup analyses suggest that this was true for dementia with a vascular component, but not for pure AD. These results were confirmed in an independent patient sample from Italy. In isolated primary cortical neurons, oxidative stress induced CCR5 expression by a NF-κB-dependent translocation mechanism. Increased cell death with activation of p53 and caspase-3 occurred in CCR5⁻/⁻ neurons exposed to oxidative stress.

Conclusions: Carriers of the ApoEpsilon4/CCR5-delta32 genotype have a seven-fold greater risk of vascular and mixed dementia and this risk increases to 10 times beyond 80 years. We propose the vulnerability of CCR5-deficient neurons in response to oxidative stress as a possible mechanism contributing to dementia occurrence.
LONG TERM BLOOD PRESSURE VARIABILITY IS ASSOCIATED TO COGNITIVE IMPAIRMENT AND MEDIAL LOBE TEMPORAL ATROPHY

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Aims: Recent evidence suggests that blood pressure variability (BPV) may be associated with cognitive impairment, independently of mean BP. Our aim was to study whether BPV between different visits (long-term BPV) was associated with cognitive decline and medial temporal lobe atrophy (MTA) in hypertensive subjects.

Methods: 361 hypertensive patients were cognitively evaluated in two visits, separated by four years. In both visits, a cerebral MRI and the Dementia Rating Scale-second edition (DRS-2) were performed and each subject’s cognitive status (MCI or normal) were defined. MTA was evaluated using the MTA-score described by Scheltens and white matter hyperintensities progression between both MRI using the Rotterdam Progression Scale. Regular BP controls obtained in Primary Care between both visits were used to calculate indexes of long-term BPV [standard deviation (SD), coefficient of variation (CV) and Average Real Variability (ARV)].

Results: In logistic regression models, increased SD (OR 1.14, CI95%1.01-1.29), CV (OR 1.19; CI95%1.02-1.40) and ARV (OR 1.11; CI95%1.01-1.22) of systolic BP was associated with incident MCI, independently of cumulative BP values. DRS-2 score was negatively related with systolic CV (β -0.03;CI95% -0.06 to -0.01) and systolic ARV (β -0.02;CI95% -0.04 to -0.02). The higher quartiles of systolic SD and CV showed higher MTA. However, there was no association between long-term BPV indexes and ischemic white matter lesions progression between both cerebral MRI.

Conclusions: In our series of hypertensive patients, long-term BPV was a predictor of cognitive decline, incident MCI and a higher degree of MTA, independently of BP levels.
Aims: Vascular dementia (VaD) is a progressive cognitive impairment caused by a reduced blood supply to the brain. Chronic cerebral hypoperfusion (CCH) is one cause of VaD; it induces oxidative stress, neuroinflammation, and blood-brain barrier (BBB) disruption, damaging several brain regions. Vitamin C plays a vital role in preventing oxidative stress-related diseases induced by reactive oxygen species, but it is easily oxidized and loses its antioxidant activity. To overcome this weakness, we have developed a vitamin C/DNA aptamer complex (NXP031) that increases vitamin C’s antioxidant efficacy. Aptamers are short single-stranded nucleic acid polymers (DNA or RNA) that can interact with their corresponding target with high affinity.

Methods: We established an animal model of VaD by permanent bilateral common carotid artery occlusion (BCCAO) in 12 week old Wistar rats. Twelve weeks after BCCAO, we injected NXP031 into the rats intraperitoneally for two weeks at moderate (200 mg/4 mg/kg) and high concentrations (200 mg/20 mg/kg).

Results: NXP031 administration alleviates cognitive impairment, microglial activity, and oxidative stress after CCH. NXP031 increased the expression of basal lamina (laminin), endothelial cell (RECA-1, PECAM-1), and pericyte (PDGFRβ); these markers maintain the BBB integrity. We found that NXP031 administration activated the Nrf2-ARE pathway and increased the expression of SOD-1 and GSTO1/2.

Conclusions: These results suggest that this new aptamer complex, NXP031, could be a therapeutic intervention in CCH-induced VaD.
Aims: Recently our lab identified a membrane ion exchanger, NHE1 (encoded by SLC9A1), known to be related to cardiovascular and cerebrovascular disease (CVD), as potentially also connected to the development of tau pathology. In this study, we pursued a pharmacologic approach to determine if NHE1 played a causative role in these processes.

Methods: We used a db/AD mouse model that we developed, a cross between the db/db diabetic mouse and the APPSwed/PS1(P264L) double knock-in model of amyloid and CVD pathology. db/AD mice and genotype controls were treated with the highly specific NHE1 inhibitor, HOE 642 (Cariporide), milled into standard chow (at 0.5% wt/wt) or control diet, for approximately 2 months. At the end, mice were subjected to behavioral tests, and multiple AD-related neuropathological endpoints were measured.

Results: NHE1 and tau expressions were related in the brain. In addition, when segregated by genotype, db/AD mice had the highest NHE1 expression compared to other subgroups. We found no indication that NHE1 influenced amyloid pathology. Treatment with Cariporide showed a notable improvement in cognitive function, as measured by Radial Arm Water Maze at the end of the study.

Conclusions: Therefore, NHE1 may be related to some aspects of neuronal injury and pathology associated with AD and related disorders. Interestingly, although Cariporide was explored as a potential therapeutic for cardiovascular injury, it failed to demonstrate efficacy in phase 3 clinical trials. Our initial studies indicate that this drug may yet retain some potential to treat some aspects of neurological disease. Funded by NIH (AG059123).
Aims: To date, no safe and effective pharmacological treatment has been clinically validated for improving stroke neurogenesis. Growth factors are good candidates but low safety has limited their application in the clinic. An additional restraint is the delivery route. The intranasal (IN) entry presents many advantages.

Methods: A brain lesion was induced in twenty-four rats. Nerve growth factor (NGF) 5 µg/kg/day, or vehicle (control group), was given IN at 10 days for two periods of five weeks, two weeks apart (wash out) to test two treatment durations. Lesion volume and atrophy were identified by magnetic resonance imaging. Anxiety and sensorimotor recovery were measured by behavior tests. Neurogenesis, angiogenesis and inflammation were evaluated by histology at 3 months.

Results: A remarkable neurogenesis and tissue reconstruction was detected in the NGF compared to vehicle group (8.1% vs 2.4%, respectively). In the new tissue, NGF significantly increased the percentage of mature neurons (19% vs 7%). Angiogenesis and inflammation were not different in the two groups. Sensorimotor recovery was not improved neither hampered by NGF during the first period of treatment but slightly decreased during the second period.

Conclusions: The first five-week period of treatment was safe while the second one highlighted a retard in recovery. The non-invasive NGF treatment can easily be transferred to the clinic; however, NGF safety profile needs to be improved for treatment longer than 5 weeks. This study is the first presenting the effects of a long treatment with NGF and has shown an important tissue regeneration rate.
Aims: The aim of this study is to investigate whether the impairment of adrenomedullin (ADM) processing, expressed as bio-ADM/mid-regional pro-adrenomedullin (MR-proADM) ratio and reduced α-amidating activity in plasma, could serve as predictive biomarkers for the preclinical stage of Alzheimer’s disease (AD).

Methods: Concentration of bio-ADM and MR-proADM in plasma was determined in 4366 subjects with no previous history of cardiovascular disease (63-75 years old, seven years follow-up, Malmö Preventive Project), using chemiluminescence immunoassay, developed by SphingoTec GmbH and BRAHMS GmbH, respectively. Amidating activity was determined in an amidation assay, as described in Kaufmann et al., 2021.

Results: The concentration of bio-ADM was decreased in patients with incident AD, when compared with AD-free population (hazard ratio [HR] = 0.73; 95% confidence interval [CI], 0.6-0.87; p=0.01). The MR-proADM concentration stayed unchanged between both groups (p>0.1). The bio-ADM/MR-proADM ratio was significantly lower in incident AD than in patients free of AD (HR = 0.66; 95% CI, 0.55-0.79; p<0.001). We could show that amidating activity in plasma was reduced in subjects with incident AD compared to disease-free individuals (HR = 0.72; 95% CI, 0.60-0.85; p=0.01).

Conclusions: The decreased bio-ADM/MR-proADM ratio in plasma is associated with incident AD and thus could serve as a predictive biomarker for the preclinical stage of AD. It is reasonable to assume, that the progression of clinical AD is connected with the C-terminal amidation, since the most significant decrease in peptide concentration in incident AD group was observed for bio-ADM only, whereas the concentration of the ADM peptide precursors were not significantly affected.
ELUCIDATING THE DIAGNOSTIC, THERAPEUTIC AND MECHANISTIC IMPLICATIONS OF STROKE IN ALZHEIMER’S DISEASE


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Aims: Alzheimer’s disease (AD) is the most common causes of dementia. In up to 90% of the cases AD is paralleled by cerebrovascular pathologies such as ischemic strokes. There is evidence that ischemic strokes can significantly increase the risk of developing dementia and vice versa. Both, AD and ischemic stroke, share the same (vascular) risk factors like age and hypertension. The exact mechanisms, how both pathologies are intertwined, have not been investigated yet.

Methods: To elucidate sex-specific interconnections of AD and ischemic stroke, we induced an ischemic stroke in male and female APPswe/PS1dE9 and C57Bl/6 wild type mice by a transient middle cerebral artery occlusion (tMCAO). Following a longitudinal paradigm, the symptomatic consequences of AD and stroke were assessed before stroke induction and three times post-stroke with a behavioral test battery (e.g. Open field, Grip test, Pole test). Furthermore, all animals were subjected to three (f)MRI scans to measure structural and functional brain connectivity (e.g, rsfMRI, DTI, FAIR-ASL). Cognitive abilities were tested during the Morris water maze at 12 months of age. Digital ventilated cages (DVC, Tecniplast) system was used to study individual locomotion via calculation of DVC metric measures (activity, distance, velocity, laterality) 24/7 before and after surgery.

Results: from the behavioral and imaging experiments will be presented and are currently being analyzed.

Conclusions: This study will help to elucidate mechanisms of stroke on AD pathophysiology and sex-specific differences. Insights will help in the development of personalized treatment strategies, dependent on the sex, age and the time window after stroke.
Aims: Mechanisms driving cerebrovascular decline during aging and in disease are poorly understood. Methylenetetrahydrofolate reductase (MTHFR) is a critical enzyme in the folate/methionine/homocysteine pathway. Variants in MTHFR, notably C677T, have been associated with AD and vascular dementia. Approximately 30% of individuals carry at least one copy of MTHFR C677T, causing a 50% decrease in MTHFR enzyme efficiency. Reduced efficiency can lead to high levels of homocysteine in blood, resulting in vascular inflammation and increased risk for cerebrovascular damage. We hypothesize that brain-specific vascular expression of MTHFR C677T in cerebrovasculature drives damaging effects.

Methods: We utilized CRISPR to create a novel knock in (KI) mouse carrying the MTHFR C677T allele on the C57BL/6J background. Liver and brain enzyme function were assessed in young MTHFR C677T mice while plasma homocysteine was measured both in young and aged cohorts. Immunohistochemistry, electron microscopy and PET/CT were used to examine cerebrovascular density, morphology and function, respectively.

Results: Mthfr C677T mice have reduced liver and brain enzymatic function and increased plasma homocysteine levels, which corresponds to human data. Young mice carrying one or two risk alleles show significantly reduced vascular density in frontal cortex as well as reduced blood perfusion in several brain regions. Cerebrovascular ultrastructure shows endothelial and pericyte apoptosis, reduced luminal size, and increased astrocyte and microglial presence in the microenvironment.

Conclusions: A novel mouse strain has been created to determine mechanisms by which the MTHFR C677T polymorphism increases risk for cerebrovascular dysfunction. This work aims to identify novel therapeutic approaches that reduce cerebrovascular pathology in AD and other dementias.
PREVALENCE OF MILD COGNITIVE IMPAIRMENT AMONG ELDERLY PEOPLE IN KAZAKHSTAN.

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Aims: Objective: To investigate the prevalence of mild cognitive impairments (MCI) among people aged 60 years and older in Almaty city, Kazakhstan.

Methods: We evaluated 668 Almaty residents aged 60 years and over using clinical and neuropsychological battery tests such as GDS, MOCA (cut-point ≤26), CHAMP Clinic Questionnaire from September 1, 2017 to October 1, 2017. A diagnosis of normal cognition, MCI, or dementia was made using standard criteria.

Results: Of the 668 participants, 662 diagnosed without dementia and 6 with dementia. Of the 662, MCI classified in 201 elderly; whereas, 461 had normal cognition. The prevalence of MCI was increased with age (p=<.0001). The overall estimated MCI prevalence in this population was 30% (95% CI: 0.26, 0.33). Elderly with lower education level and those who had a history of brain injury had higher odds having MCI.

Conclusions: The prevalence of MCI among elderly population in Almaty, Kazakhstan is 30%. Aging, less years of education, and brain injury were associated with MCI.
Aims: Today, about two million elderly people live in Kazakhstan, which is more than 10% of the population, thus crossing the seven percent threshold for the definition of an “aging” country in the world. Globally, mild cognitive impairment (MCI) prevalence ranges from 3% to 42%. Dementia syndrome can develop in 38% of patients with MCI within 5 years. Purpose of the study: to study the clinical and epidemiological features of cognitive impairment in the Kazakh population and to develop a set of measures for the differential prevention of dementia, taking into account the ethnic characteristics of the prevalence of the main risk factors cognitive decline.

Methods: Materials and methods. The screening involved 474 respondents aged 60 and over, Kazakhs, 136 men, 338 women, average age 69.6. Material collection was carried out using the Champ Clinic Questionnaire and the MOCA test (cut-off point <26), a short scale for assessing depression based on 6 polyclinics in Almaty.

Results: There was a high prevalence of diseases: 52.6% had arterial hypertension, 18% had a stroke, and 25.2% had diabetes. Traumatic brain injuries were very common in the anamnesis (36%). The average score on the MoCA scale was 21.5. The prevalence of MCI was 30.4% among Kazakhs, 31.7% in the main group.

Conclusions: New data have been obtained on the prevalence and clinical features of cognitive impairments in the Kazakh population, which will make it possible to develop a system of differentiated prevention of cognitive decline, taking into account ethnic and gender characteristics of the leading modifiable risk factors.
POSTERS

SINGLE CELL RNA SEQUENCING AS A NOVEL STRATEGY FOR UNCOVERING THE MECHANISMS OF ALZHEIMER’S DISEASE

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Aims: The failures of clinical trials for Alzheimer’s disease (AD) highlight that deeper understanding of AD disease mechanisms is needed. The brain is the most complex organ in the body and its diversity of cell types and extreme sensitivity to cellular stress have made it difficult to study. Single-cell RNA sequencing (scRNAseq) has emerged as the most comprehensive method for identifying changes in the transcriptome of individual cells but is costly and difficult to reliably establish. Our lab is using this technology to uncover new mechanisms that can be targeted in AD.

Methods: Our new established dissociation protocol yields high viability and high diversity of brain cell populations, key steps to studying the aging brain and its dysfunction in AD. With this advancement, we have combined it with a new platform for Single-cell RNA sequencing, the BD Rhapsody. This platform allows us to multiplex multiple samples simultaneously to reduce sequencing batch effects and maximize efficiency.

Results: By analyzing dissociated brain regions of interest such as the hippocampus, hypothalamus, and the sub-ventricular zone of 3xTg-AD and WT mice, we were able to identify cell-specific gene expression profiles associated with AD. Key biological processes differed among specific cell populations between the 3xTg-AD and WT mice, relating to lipid metabolism, immune response, and vascular function of the blood brain barrier. We are currently exploring in greater depth bio-informatically.

Conclusions: We have validated an optimized dissociation protocol compatible with the BD Rhapsody platform which can advance research on aging, Alzheimer’s disease, and other neurological conditions.
Aims: Vascular dementia (VaD) is the second-most common form of dementia. Although the pathobiology of VaD is poorly understood and lacks effective treatment, clinically relevant factors such as age and sex are rarely considered in preclinical modeling. This places efforts to develop future therapies at a severe disadvantage, as clinically relevant translation of underlying pathobiology may be lost.

Methods: We reviewed common physiological and morphological and outcomes in six major rodent models of VaD in 258 full-text publications: 1) chronic cerebral hypoperfusion, 2) high fat diet, 3) diabetes, 4) hypertension, 5) carotid arterial calcification, and 6) CADASIL, and evaluated translational features relevant to human pathophysiology with an analysis of timepoints of observed pathology and rodent sex and age.

Results: All models of VaD shared common features of decreased CBF and upregulation of inflammatory signaling molecules such as TNF-α, IL-1β, and IL-6, and reactive oxygen species (ROS) such as SOD and NOX, consistent with clinical presentations of VaD. Only the carotid artery calcification model showed inconsistent evidence for brain endothelial tight junction loss, astrogliosis, and macrophage reactivity, in disagreement with other models.

Conclusions: Improved translational insight may critically depend on 1) inclusion of a constellation of comorbidities in preclinical modeling instead of modeling each in isolation to better capture human disease pathogenesis, 2) increased use of middle-aged and aged preclinical rodent models which are sensitive to clinically relevant disease presentation, and 3) inclusion of female animals in models as sex is a relevant biological factor in better defining future therapeutic strategy in real-world populations.
LOSS OF PRION PROTEIN CONTROL OF GLUCOSE METABOLISM CONTRIBUTES TO NEURODEGENERATION IN PRION DISEASES.

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Aims: Corruption of cellular prion protein PrPC neuronal function(s) leads to prion diseases, such as Creutzfeldt-Jakob disease. The roles exerted by PrPC, however, remain partially understood. We aimed to identify cellular functions governed by PrPC and investigate whether dysregulation of those functions contribute to prion diseases.

Methods: To grasp PrPC roles, we compared the proteome and metabolome of PrPC expressing 1C11 neuronal cells to those of PrPnull-1C11 cells stably repressed for PrPC expression. The status of novel cellular functions governed by PrPC was then probed in prion-infected mice.

Results: We show that PrPC contributes to the regulation of the energetic metabolism by orienting cells towards mitochondrial oxidative degradation of glucose. Through its coupling to cAMP/protein kinase A signaling, PrPC tones down the expression of the pyruvate dehydrogenase kinase 4 (PDK4) and optimizes the activity of the pyruvate dehydrogenase complex. Such PrPC action not only favors the transfer of cytosolic pyruvate into mitochondria and its conversion into acetyl-CoA, but also limits fatty acid b-oxidation and subsequent onset of oxidative stress conditions. The corruption of PrPC metabolic regulatory role by pathogenic prions PrPSc causes in the mouse brain an imbalance between glucose oxidative degradation and fatty acid b-oxidation in a PDK4-dependent manner. The inhibition of PDK4 counteracts PrPSc-induced metabolic derangements and extends the survival of prion-infected mice.

Conclusions: Our study reveals a new role of PrPC in the control of the glucose energetic metabolism whose dysregulation by PrPSc contributes to prion diseases. Our data further introduce PDK4 as a potential therapeutic target to combat prion diseases.
POSTERS

BLOOD-BASED PRONOSTIC BIOMARKERS FOR PARKINSON’S DISEASE PROGRESSION AND SEVERITY

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Aims: Determine a physiopathological panel of blood-based prognostic markers, significantly associated with the severity and the disease progression of Parkinson’s disease.

Methods: 560 sera samples were collected as part of a large prospective multi-centric cohort of PD patients at the stage of severe motor fluctuations (PREDISTIM). Clinical parameters such as age, disease duration, MDS-Unified Parkinson’s Disease Rating Scale (MDS-UPDRS) to score motor and non-motors symptoms, Montreal Cognitive Assessment (MoCA) to score cognitive impairment and the Parkinson’s Disease Questionnaire (PDQ-39) to assess quality of life were used as proxy of severity and disease progression to assess the prognostic value of candidate blood-based biomarkers. Markers for neuronal integrity (Neurofilament Light Chain (NfL)), lipid peroxidation (4-hydroxy-2-nonenal (4-HNE), Glutathione Peroxidase (GPx) activity), iron status (ferritin), inflammation (Interleukine-6 (IL-6), Tumor Necrosis Factor-alpha (TNF-α)) and protein aggregation (α-synuclein) were assessed by electrochemiluminescence, multiplex fluorescence or ELISA.

Results: NfL, 4-HNE, IL-6 and α-synuclein were positively correlated with MDS-UPDRSIII worst-off score and MDS-UPDRS total score; NfL, IL-6, TNF-α and α-synuclein were positively correlated with Hoehn & Yahr in univariate analysis with adjustment on age and disease duration. Also, NfL and α-synuclein were positively correlated with MDS-UPDRS total score and TNF-α correlated with Hoehn & Yahr in multivariate analysis.

Conclusions: NfL, TNF-α and α-synuclein are three biomarkers we identified as predictors of the severity of Parkinson’s disease. Further analysis on longitudinal data and other cohorts of patients need to be assessed to confirm their prognostic value and their potential use in clinical trial.
POSTERS

MICRORNA BIOGENESIS DEFICITS IN HUNTINGTON'S DISEASE

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Aims: Alterations in microRNA (miRNA) expression is a common feature of Huntington’s disease (HD) and could participate in disease onset and progression. So far, however, little is known about the underlying causes of miRNA disruption in HD. We and others have recently shown that Ago2, a major component of the miRNA biogenesis pathway, goes awry in HD mice overexpressing mutant Huntington (mHTT). We therefore sought to evaluate in detail miRNA biogenesis in human HD.

Methods: We characterized all major miRNA biogenesis pathway components and miRNA maturation products (pri-miRNA, pre-miRNA, and mature) in human HD (N=41, Vonsattel grades HD2-4) and healthy control (N=25) subjects. Notably, the striatum and cortex from the same individuals were analyzed in parallel.

Results: We show that Ago2, Drosha, and Dicer were strongly downregulated in HD at early stages of disease. Using a panel of HD-related miRNAs (miR-10b, miR-196b, miR-132, miR-212, miR-127, miR-128), we uncovered various types of maturation defects in HD brain, the most prominent occurring at the pre-miRNA to mature miRNA maturation step. We provide evidence that autophagy could participate in this process. Notably, most changes occurred in the striatum that is more prone to HTT pathology and neurodegeneration. Lastly, we observed no significant alterations in miRNA biogenesis in human HD blood, and variable effects in a transgenic HD mouse model (R6/2).

Conclusions: These fundings provide important clues into the underlying mechanisms behind miRNA alterations in human HD. Further investigations are now required to understand the biological, diagnostic, and therapeutic implications of miRNA/RNAi biogenesis defects in HD and related neurodegenerative disorders.
ASSOCIATION WITH PROTEASOME DETERMINES PATHOGENIC THRESHOLD OF POLYGLUTAMINE EXPANSION DISEASES

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Aims: Expansion of glutamine residue track (polyQ) within soluble protein is responsible for eight autosomal-dominant genetic neurodegenerative disorders. These disorders affect cerebellum, striatum, basal ganglia and other brain regions. Each disease develops when polyQ expansion exceeds a pathogenic threshold (Qth). A pathogenic threshold is unique for each disease but the reasons for variability in Qth within this family of proteins are poorly understood. In the previous publication we proposed that polarity of the regions flanking polyQ track in each protein plays a key role in defining Qth value. To explain the correlation between the polarity of the flanking sequences and Qth we performed quantitative analysis of interactions between polyQ-expanded proteins and proteasome.

Methods: Bioinformatics and modelling

Results: Based on structural and theoretical modeling, we predict that Qth value is determined by the energy of polar interaction of the flanking regions with the polyQ and proteasome. More polar flanking regions facilitate unfolding of α-helical polyQ conformation adopted inside the proteasome and as a result, increase Qth.

Conclusions: Predictions of our model are consistent with Qth values observed in clinic for each of the eight polyQ-expansion disorders. Our results suggest that the agents that can destabilize polyQ α-helical structure may have a beneficial therapeutic effect for treatment of polyQ-expansion disorders.
POSTERS

MEDIUM SPINY NEURON-SPECIFIC RECEPTOR GPR55 IS DOWNREGULATED IN PARKINSON’S DISEASE AND MODULATES STRIATAL EXCITABILITY

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Aims: GPR55 is expressed specifically in human striatum and so has therapeutic potential for Parkinson’s, Huntington’s and epilepsy for example. Studies aimed at understanding its role in these diseases have been hampered by the absence of selective modulators that are active across species. Our aim was to develop GPR55 activators and functional antagonists with cross-species potency and to explore changes in GPR55 expression in disease context.

Methods: Using Cerevance’s proprietary NETSseq technology and post-mortem human brain samples from human Parkinson’s disease (PD) and control donors (non-CNS-related cause of death), we produced a deep cell type-specific transcriptomic analysis from multiple brain regions. Compounds were tested on recombinant Ca2+ flux and β-arrestin assays and investigated in neuroprotection assays and electrophysiologically on rat MSNs.

Results: GPR55 is preferentially expressed in human striatal MSNs and downregulated in MSNs from PD compared to non-affected controls. Novel activators of GPR55 were optimised to achieve high potency at rodent and human receptors and excellent brain permeability. GPR55 coupling via G12/13 was confirmed and degree of β-arrestin recruitment was correlated with receptor desensitisation. In patch clamp studies, compounds decreased the frequency of rat MSN cell firing. In rat striatal glutamate-toxicity assay systems a partial rescue of neurite retraction was observed with agonists that do not recruit β-arrestin.

Conclusions: We have developed the first nanomolar rodent GPR55 agonists with differential receptor desensitisation profiles. These molecules show therapeutic potential by modulating firing of striatal MSNs and providing neuroprotection in a glutamate-injury model.
EVALUATING THE THERAPEUTIC POTENTIAL OF ENDOGENOUS GDNF IN PROTEASOME INHIBITOR LACTACYSTIN MOUSE MODEL OF PARKINSON'S DISEASE

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Aims: Parkinson’s disease (PD) is a progressive neurodegenerative disease. The classical motor symptoms of PD mostly result from a gradual degeneration of Substantia nigra pars compacta dopaminergic neurons and the consequent loss of dopamine in the dorsal striatum. Neurotrophic factor GDNF promotes the survival of dopamine neurons in vitro and in vivo. Intracranial delivery of GDNF has been tested in clinical trials for treating PD; however, ectopic delivery of GDNF results in highly variable treatment and side effects. To overcome such problems, our laboratory has shown that it is possible to modulate GDNF expression levels without altering spatial expression patterns by editing the 3’ untranslated region (3’UTR) of a Gdnf gene. We found that already a two-fold constitutive increase in endogenous GDNF expression in GDNFHypermorphic mice has a beneficial effect in Parkinson’s disease mouse model without causing the typical side-effects associated with ectopic GDNF expression.

Methods: In this study, we comprehensively studied the therapeutic potential of endogenous GDNF upregulation using our new conditional GdnfHyper allele in experimental paradigms evaluating both neuroprotection and neurorestoration.

Results: Our results definitively show that upregulation of endogenous GDNF in the adult striatum is not protective in the proteasome inhibitor lactacystin (LC) induced PD model in mice. We also analyze the effect of deletion of endogenous GDNF using Gdnfconditional Knock Out allele in aged mice and reveal that GDNF deletion does not increase susceptibility to LC-induced damage.

Conclusions: Altogether, our results show that endogenous GDNF does not impact the outcome in the LC-induced mouse model of PD.
SUB-ANESTHETIC KETAMINE-TREATMENT LEADS TO IMPROVED MOTOR BEHAVIOR AND AFFECTS MICROGLIAL MORPHOLOGY IN THE PROGRESSIVE UNILATERAL 6-OHDA LESION RAT MODEL

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**Aims:** Using a 6-hydroxydopamine (6-OHDA) Parkinson’s disease (PD) rat model, our lab has shown that sub-anesthetic ketamine is acutely anti-parkinsonian and has long-term anti-dyskinetic effects, the latter of which is brain-derived neurotrophic factor (BDNF) dependent. Here, we investigate if sub-anesthetic ketamine may additionally exhibit neuroprotective effects in a progressive 6-OHDA rat model of PD.

**Methods:** Male Sprague-Dawley rats were treated with either ketamine or vehicle (6-hr treatment; 3x20 mg/kg; i.p.; 2-hrs apart) prior to unilateral intrastriatal 6-OHDA lesion, and for 7 days post-lesion. Using the amphetamine-induced rotation test, asymmetry was assessed by counting the net ipsilateral rotations (mean±SEM) over 90-minutes.

**Results:** On Day 28 post-lesion, ketamine (211±116) led to a significantly decreased number of ipsilateral rotations compared to vehicle (666±187; two-tailed t-test; p<0.05; n=15). Unbiased stereology of dopaminergic neurons in the substantia nigra (SN) is ongoing and we are investigating the role of BDNF in ketamine’s neuroprotective effects via in situ hybridization for BDNF and its receptor, TrkB. To investigate anti-inflammatory action, we stained SN and striatum with the microglia marker, IBA1, and analyzed branch length/cell and end points/cell. We found no effects in the SN, but the number of end points/cell in the striatum was significantly reduced by 6-OHDA in vehicle-treated rats (p<0.001; n=8), but not in those treated by ketamine. Additional assessment of a functional marker of microglia (CD68) is ongoing.

**Conclusions:** These data indicate that sub-anesthetic ketamine affects microglial morphology and is neuroprotective, further supporting the currently ongoing clinical trials in individuals with PD who developed LID.
Aims: Neurotrophins are growth factors indispensable for brain development and function. Likewise, neurotrophins dysregulation was observed in a variety of neurodegenerative disorders, this makes them ideal drug candidates for diseases treatment. Several issues have to be overcome for the successful clinical translation of neurotrophins. Here, we propose the use of cyclic peptide mimicking nerve growth factor (cNGF), characterized by specific receptor targeting, improved bioavailability and low degree of proteolysis, as a suitable and reliable strategy for neuroprotection, assisted by copper interaction.

Methods: Firstly, we characterized the chemical structure/properties and the TrkA binding capacity of cNGF by molecular dynamics and docking simulations, NMR and enzymatic hydrolysis assay. In addition, copper interaction was investigated by potentiometric, spectroscopic and EPR measurements. Secondly, different biochemical approaches including western blot, ELISA and immunofluorescence imaging analysis, were carried out to evaluate the cNGF neurotrophic activity on PC12 cell line.

Results: Our results demonstrated that cNGF exerts neuroprotective function by interaction and phosphorylation of TrkA, activation of CREB-signaling, induction of cell differentiation and BDNF, VEGF protein release. In addition, for the first time, we demonstrated that cNGF also phosphorylates VEGFR1 and VEGFR2 receptors. Intracellular copper homeostasis resulted to be affected by peptide cNGF treatment. Consistently, interaction between copper and cNGF was evident as the addition of copper chelator BCS leads to markedly decrease the effect of peptide.

Conclusions: Overall, these findings support the hypothesis that neurotrophin cyclic peptides may be more effective therapeutics for neurodegenerative diseases, respect to linear peptidomimetic analogues previously investigated, by regulating different intracellular pathways and copper homeostasis.
Aims: The Hippo pathway (HP) promotes cell survival and proliferation. It consists in cascade of kinases which controls phosphorylation of the co-activators YAP/TAZ. When phosphorylated YAP/TAZ translocate into the nucleus, where they mainly bind to the TEAD transcription factor family and activate genes related to cell proliferation and survival. HP involvement recently emerged in neurodegenerative diseases such as Huntington’s disease (HD) or Alzheimer’s disease (AD) and targeting the HP might be beneficial in murin models of these disorders. The project aims explore an innovative therapeutic strategy dedicated to increase activation of TEAD based on the modulation of HP with these molecules in different neurodegenerative diseases.

Methods: We tested several molecules using a TEAD reporter Luciferase assay to select activators of TEAD transcriptional activity. Then we evaluated selected drugs for their capability to increase YAP/TAZ-TEAD target genes (RT-qPCR and WB) on different cell types and measured the effects of selected drugs on the proliferation (Incucyte).

Results: One of the activators inhibits Last1/2. At 10 µM, it activates the TEAD transcriptional activity by 80%. It increases RNA and protein expression of YAP/TAZ-TEAD target genes Cyr61 and CTGF, in HEK293T cells and small molecule neuronal progenitor cells (smNPC). It induces an increase of cell proliferation of HEK293T cells after 24h of treatment as well as in smNPC (to be confirmed).

Conclusions: We found a promising compound in modulating the HP increasing cell proliferation and the expression of target genes linked to cell survival and proliferation. In perspective, we will test it in other cellular models including HD.
NEUROPROTECTIVE EFFECT OF EXTRACELLULAR MITOCHONDRIA AGAINST FERROPTOSIS

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Aims: Mitochondrial dysfunction plays an important role in both ferroptosis and neurodegenerative disease. Preserving mitochondrial function is a promising approach to prevent ferroptosis and cure neurodegenerative disease. Recently, mitochondrial transplantation has been demonstrated to effectively improve neuronal survival by directly transferring exogenous healthy mitochondria to damaged cells. In this study, we intend to obtain mitochondria from healthy neurons, apply them to cells treated with different ferroptotic inducers (Erastin, glutamate, and RSL3), then examine their neuroprotective potential in ferroptosis.

Methods: Mitochondria were isolated from healthy neuronal HT22 cells by homogenization and centrifugation steps. Isolated mitochondria were co-treated with HT22 cells in the presence or absence of ferroptotic stimuli. Mitochondrial incorporation was observed through MitoTracker green labeling followed by live imaging and flow cytometry. Metabolic changes were determined by MTT assays. Cell death was evaluated by propidium iodide staining and flow cytometry.

Results: Live imaging and flow cytometry analysis demonstrated the incorporation of exogenous mitochondria into both healthy and damaged cells. Remarkably, we observed more incorporated mitochondria in HT22 cells damaged by erastin. MTT assays indicated incorporated mitochondria significantly improved cell viability against ferroptosis in a dose dependent manner. Flow cytometry results further confirmed mitochondrial transplantation can decrease the number of dead cells. This neuroprotective effect was decreased by inhibitors of mitochondrial complex I and V.

Conclusions: In the present study, we confirmed the incorporation of exogenous mitochondria, which is closely associated with rescuing neuronal cells from ferroptosis. The neuroprotection mediated by exogenous mitochondria was highly dependent on intact activity of mitochondrial complex I and ATP synthase.
THE ROLE OF GANGLIOSIDES IN THE SECRETION OF EXTRACELLULAR VESICLES AND MUTANT HUNTINGTIN

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Aims: Gangliosides are glycosphingolipids involved in cell signaling and cell-cell interactions. In Huntington's disease (HD), levels of ganglioside GM1 are reduced. GM1 replenishment in HD mouse models corrects both motor and non-motor symptoms while significantly reducing levels of both soluble and aggregated mutant huntingtin (mHTT) through a mechanism independent of Htt gene transcription. We aimed to identify mHTT-lowering pathways induced by GM1 using proteomics. Based on our results, we investigated how gangliosides promote mHTT clearance through extracellular vesicle (EV) secretion.

Methods: To identify the targets of GM1, we performed an unbiased label-free proteomics screen on wild-type or knock-in Q140 HD mice administered artificial CSF (vehicle) or GM1 by intraventricular infusion for 42 days. Proteins from the striatum and cortex were analyzed by Gel-LC-MS/MS. Differentially expressed proteins were identified using a linear model and two-way ANOVA. EVs were analyzed using a combination of imaging flow cytometry, fluorometry and immunoblotting.

Results: The EV Gene Ontology cell compartment term was highly represented in our proteomics dataset. EVs are membrane-enclosed nanoparticles that participate in cell-cell communication and proteostasis. We confirmed that GM1 significantly elevated EV secretion from WT and mHTT-expressing neuronal cells and enhanced mHTT elimination through EVs. Furthermore, we found that cellular GM1 levels positively correlate to EV secretion.

Conclusions: Altogether, our data identify gangliosides as novel modulators of EV secretion and suggest that GM1-induced EV secretion may contribute to the mHTT-lowering effect of the ganglioside in HD. These findings potentially have relevance to other protein misfolding diseases where ganglioside levels are perturbed, including Parkinson's disease.
THE EXPERIMENT OF THE MILITARY CENTRAL HOSPITAL’S NEUROSURGERY DEPARTMENT IN THE DBS

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Aims: Faced with the significant increase in the number of consultations in neurology at the level of our structure, for the management of Parkinson's disease and especially the forms refractory to medical treatment, in the sense that the side effects of therapy have become incompatible with a self-sufficient and decent daily life; which were originally transferred overseas for deep brain stimulation (DBS), we have decided to take care of them at our level, ie in neurosurgery of the same structure.

Methods: Since 2011 we have taken care of (08) eight patients including (07) male and one female. The treatment consisted of the installation of a deep brain stimulation device in (07) cases including (06) cases of Parkinson's disease and (01) case of abnormal movements in which we proceeded to a change of the stimulator after 04 years. Another patient treated for Parkinson's disease also received a replacement of his pacemaker after 06 years of performance.

Results: The postoperative complications observed are: pneu cephalus, headaches and depression in one case each, hyper sexuality and aggressivity in one case as well. Therefore, our results can be superimposed on all the major series, as clinical improvement and socio-professional reintegration were obtained in all cases.

Conclusions: All things considered, it is strongly recommended to manage this pathology surgically as soon as the indication is there, reckoning that the patients' selection suggested a multidisciplinary approach: neurology, neurosurgery, psychiatry, neuropsychology, and the final decision is made based on evaluations by each discipline.
Aims: To evaluate the effects of early environmental enrichment (EE) exposure (from postnatal day 21 to postnatal day 60) on behavioral and brain monoamine levels in the YAC128 HD mice.

Methods: Male and female wild-type and YAC128 mice were exposed to a controlled environment or EE for 39 days. A battery of behavioral tests was applied at two months of age in the following order: elevated plus maze, pole, open field, splash, tail suspension, and accelerating rotarod tests. After the last test, the hippocampus, prefrontal cortex, and striatum were dissected to measure monoamine levels by high-performance liquid chromatography (HPLC). The data were analyzed using two-way ANOVA followed by the Tukey post hoc test. Results were expressed as mean ± standard deviation (SD). The correlation between latency to the first fall and NA striatum levels was performed using Pearson’s r correlation test for the YAC128 (CE and EE) group. A p-value of ≤0.05 was statistically significant.

Results: Differences between YAC128 and WT mice were found in body weight, and motor performance in the accelerating rotarod test and EE exposure reversed these alterations. Animals exposed to EE showed decreased striatal norepinephrine levels. In addition, a significant correlation between better motor performance and lower norepinephrine levels in the striatum and hippocampus and lower serotonin levels in the striatum was found.

Conclusions: Our results confirmed motor deficits and body weight gain in YAC128 mice and showed EE reversed these impairments and modulated monoamine levels. Therefore, supporting environmental interventions is a beneficial approach to delay HD onset manifestation.
ASSESSMENT OF ABNORMAL INVOLUNTARY MOVEMENTS ACCORDING TO THE SCALE IN PATIENTS WITH ANTIPSYCHOTIC-INDUCED TARDIVE DYSKINESIA: A PILOT STUDY

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Aims: Assessment the prevalence of antipsychotic-induced tardive dyskinesia (AITD) by the Abnormal Involuntary Movement Scale (AIMS) in patients with schizophrenia.

Methods: We observed 76 patients with F20 (ICD-10): 48 (63.1%) male; 28 (36.9%) female. All patients have taken AP monotherapy of 1st and 2nd generations. We uses AIMS for the diagnosis of AITD during for 8 weeks. 51 patients were excluded from our study due to a change AP-monotherapy or for other reasons.

Results: At 1st visit, 14 patients (51.8%) had AITD: 4 (14.8%) patients – minimal severity; 9 (33.3%) - mild severity; 1 (3.7%) - severe. Hyperkinesis involving facial muscles was observed in 9 patients (33.3%), jaws - 5 (18.5%), tongue - 16 (59.2%), upper limbs - 2 (7.4%), lower limbs - 3 (11.1%), trunk - 1 (3.7%). 1 (3.7%) patient had disabilities due to AITD, and 2 (2.7%) patients were aware of the presence of AITD. At 2nd visit, 14 patients (51.8%) had AITD: 10 (37.0%) patients – minimal severity; 2 (7.4%) - mild; 2 (7.4%) - moderate severity. Hyperkinesis involving facial muscles was observed in 7 patients (25.9%), jaws - 2 (7.4%), tongue - 15 (55.5%), upper limbs - 2 (7.4%), lower limbs - 1 (3.7%), trunk - 1 (3.7%). After 8 weeks, none of the patients showed disability due to AITD, and 1 (3.7%) patient was aware of AITD.

Conclusions: The results of our pilot study demonstrated that the frequency of AITD in Russian schizophrenic patients is 53.9%. The AIMS can be used as screening scale for AITD in schizophrenia patients.
Aims: Keap1-Nrf2 pathway plays a pivotal role in the cellular defense system against oxidative stress by inducing antioxidant and anti-inflammatory effects. In this study, we designed and synthesized a class of halogenated vinyl sulfones by inserting halogens and pyridine to maximize Nrf2 activation efficacy.

Methods: Optimization of potency on Nrf2 was achieved through SAR data collected during our hit-to-lead medicinal chemistry on the key structure of vinyl sulfone that contributed to the efficacy of the previously developed compounds. These optimizations included the following: (1) the halogen substitution in the ortho position of the benzene ring, (2) substitution of the vinyl group in beta-position with a pyridine ring, and (3) insertion of a halogen in R² on pyridine ring.

Results: Among the synthesized compounds, 9d remarkably increased Nrf2 nuclear translocation and Nrf2 protein levels in microglial BV-2 cells. 9d was shown to induce the expression of antioxidant response genes at both the mRNA and protein levels and suppress proinflammatory cytokines and enzymes. Also, 9d remarkably protected DAergic neurons and restored the PD-associated motor dysfunction in the MPTP-induced mouse model.

Conclusions: The most promising 9d was identified and it showed excellent profile: (1) the molecular size was below 400 Da, (2) potent Nrf2 activation efficacy at nanomolar concentrations, and (3) favorable drug-like properties. 9d significantly increased Nrf2 levels, upregulated antioxidant enzymes, and suppressed inflammatory response. Furthermore, 9d attenuated the motor deficiency and rescued the loss of DAergic neurons SNpc and striatum in MPTP-induced mice. Collectively, 9d is presented as an improved candidate for therapy against PD.
LONGITUDINAL BRAIN AGE GAP AND COGNITIVE DECLINE AFTER STROKE

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Aims: Advanced age is associated with poorer prognosis following stroke. Machine learning based on brain imaging can be used to estimate the age of a patient and compute the difference to chronological age; the brain age gap (BAG). Higher BAG has been found in a range of clinical conditions and has been associated with increased risk of dementia and mortality. Few studies have been conducted on its association with stroke, and the predictive value for post-stroke cognitive decline and dementia is unknown. To this end, using longitudinal data after stroke we tested the hypothesis that cognitive decline after stroke is associated with a higher BAG.

Methods: 270 stroke survivors (age = 71.1 (11.0), women = 55.6%) were included from the ‘Norwegian Cognitive Impairment After Stroke (Nor-COAST) study. Clinical-, neuropsychological- and MRI data was collected shortly after the acute stroke and at 18- and 36 months follow-up. Freesurfer anatomical segmentation was conducted, and BAG computed.

Results: Mean (SD) Montreal Cognitive Assessment (MoCA) scores were 24.0 (4.6) at baseline, 24.9 (4.3) at 18 months and 24.6 (5.7) at 36 months. A significant relationship was found with global BAG at baseline and 18 months, and with right hemisphere BAG at 18 months.

Conclusions: We found that a higher global BAG was associated with worse cognition at baseline and 18 months. Higher right hemisphere BAG was also found to be associated with worse cognitive outcome 18 months after a right-sided stroke. These findings suggests that BAG may be used as a predictive marker for cognitive decline after stroke.
THE USE OF EEG BIOMARKER IN EVALUATING THE ANTI-DYSKINETIC EFFECT OF DRUGS IN PARKINSON'S DISEASE.

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Aims: In Parkinson’s disease (PD), motor symptoms result from a dysfunction of the cortico-basal ganglia circuits due to the dopamine loss. Using Electroencephalography (EEG) techiniques, a hyper synchronization of the beta rhythms in the cortico-basal ganglia loop has been observed in both parkinsonian patients and animal models and has been positively correlated to motor symptoms. This aberrant excessive beta oscillation is suppressed by dopaminergic treatments or deep brain stimulation paradigms, which simultaneously improve motor deficits. However, a chronic L-DOPA treatment induces dyskinesia and a prominent resonant in another frequency band, the gamma oscillation. This project aimed at investigating the effect of the antidyskinetic drug amantadine, which is routinely used in the clinic, on L-DOPA-induced gamma oscillations in the 6-OHDA rat model of PD.

Methods: Unilaterally 6-OHDA-lesioned rats were implanted with a bipolar electrode in the motor cortex ipsilateral of the lesion. EEG recordings were performed in freely moving animals before and after the chronic injection of L-DOPA.

Results: In this poster, we will show that chronic administration of L-DOPA at low dose (6mg/kg) induces specific gamma oscillations and dyskinesia which gradually increase with repeated treatments. We will demonstrate that a treatment with amantadine dose-dependently reduces L-DOPA-induced gamma oscillations and AIMs score.

Conclusions: Our data will illustrate how the preclinical study of cortical beta and gamma oscillations offers relevant and translatable EEG biomarkers that add significant value to drug development as stable, quantitative, and objective endpoints for the development of new antiparkinsonian and antidyskinetic neurotherapeutics.
Aims: To evaluate idiopathic normal-pressure hydrocephalus (INPH)-related cerebral blood flow (CBF) abnormalities and to investigate their relation to cortical thickness in INPH patients.

Methods: We investigated cortical CBF utilizing surface-based early-phase $^{18}\text{F}$-florbetaben (E-FBB) PET analysis in 2 groups: INPH patients and healthy controls. All 39 INPH patients and 20 healthy controls were imaged with MRI, including 3-dimensional volumetric images, for automated surface-based cortical thickness analysis across the entire brain. A subgroup with 37 participants (22 INPH patients and 15 healthy controls) that also underwent $^{18}\text{F}$-fluorodeoxyglucose (FDG) PET imaging was further analyzed.

Results: Compared with age- and gender-matched healthy controls, INPH patients showed statistically significant hyperperfusion in the high convexity of the frontal and parietal cortical regions. Importantly, within the INPH group, increased perfusion correlated with cortical thickening in these regions. Additionally, significant hypoperfusion mainly in the ventrolateral frontal cortex, supramarginal gyrus, and temporal cortical regions was observed in the INPH group relative to the control group. However, this hypoperfusion was not associated with cortical thinning. A subgroup analysis of participants that also underwent FDG PET imaging showed that increased (or decreased) cerebral perfusion was associated with increased (or decreased) glucose metabolism in INPH.

Conclusions: A distinctive pattern of CBF changes was found in INPH patients. We cautiously suggest that hyperperfusion in INPH may result from neuroinflammation and reactive gliosis. Further, we can cautiously hypothesize that hypoperfusion without cortical thinning may also be a neurodegenerative penumbra in INPH.
GENETIC SPARSE LABELING TO ILLUMINATE THE MORPHOLOGY AND PATHOLOGY OF DISEASE-RELEVANT CELL TYPES IN ALZHEIMER’S AND HUNTINGTON’S DISEASE MOUSE MODELS

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Aims: Alzheimer’s disease (AD) and Huntington’s disease (HD) are age-related neurodegenerative disorders with no disease-modifying therapies. Our current understanding of neuronal morphological degeneration is incomplete due to challenges visualizing and quantifying the detailed morphology of disease-vulnerable neurons. The goal of this study is to apply a novel genetic sparse labeling method to characterize the morphology of relevant cell types in AD and HD mouse models.

Methods: We generated a series of novel Cre-dependent membrane-bound reporter mouse lines, utilizing an out-of-frame mononucleotide repeat that can undergo frameshift mutation to sparsely and stochastically label the complete morphology of genetically-defined neurons and glia (Veldman et al., 2020, PMID: 32795398). Mononucleotide Repeat Frameshift (MORF) 3 mice were crossed with neuronal and microglial Cre mouse lines in 5xFAD and Q140 huntingtin knockin mouse models of AD and HD, respectively. The labeled brain cells were imaged in tissue-cleared thick sections or intact brain hemispheres.

Results: We developed a tissue-clearing and imaging pipeline with confocal or light-sheet microscopy to visualize the complete morphology of individual cells to the resolution of dendritic spines, axons, and endfeet. An informatic pipeline was established to process, reconstruct, and analyze high-dimensional, quantitative morphological datasets. Our analysis revealed distinct medium spiny neuron morphological defects in Q140 mice compared to wildtype controls. Our current efforts focus on analyzing cortical pyramidal neurons and microglia in 5xFAD.

Conclusions: MORF3 is a novel and powerful mouse genetic tool that illuminates the brainwide morphology of genetically-defined, disease-relevant neurons and glia, which could facilitate the study of disease modifying mechanisms and therapeutics.
POSTERS

QUANTITATIVE ANALYSIS OF POSTURAL SWAY IN SPORADIC ADULT-ONSET ATAXIA

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Aims: This study aimed to assess the overall movement of the upper body, including the head (vertex), upper (C7), and lower back (L5) in the postural sway during stance tasks in patients with sporadic adult-onset ataxia (SAOA) using an inertial measurement unit.

Methods: Nineteen patients clinically diagnosed with SAOA and 10 healthy controls have participated in this study. Static balance was measured using an inertial measurement unit system in both groups.

Results: For patients with SAOA, the average sway area in the head, upper, and lower back was significantly greater than the healthy control group in all stance tasks (stance open or closed, both with eye open or closed). The postural sway was significantly greater in the head than in the lower back. The postural sway in the head and upper back was positively correlated with ataxia severity as assessed using the scale for the assessment and rating of ataxia (SARA) and disease duration in patients with SAOA.

Conclusions: This study showed the sway area in the head and upper back is effective in quantifying the postural imbalance than the lower back in SAOA patients.
CLASSIFICATION OF ALZHEIMER’S DISEASE AND MILD-COGNITIVE IMPAIRMENT BASE ON FUNCTIONAL BRAIN CONNECTIVITY AT DIFFERENT FREQUENCY BAND AND MODULAR-LASSO FEATURES SELECTION

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Aims: Functional brain connectivity networks obtained from resting-state functional magnetic resonance imaging have been extensively utilized for diagnosis of Alzheimer’s disease. However traditional network analysis technique only explores limited relation which may not be suitable for revealing sufficient and proper functional connectivity link among brain regions.

Methods: To solve this problem, we utilized a modular-LASSO features selection scheme that can harness the modularity information to find interpretable and discriminative biomarkers form functional networks for computer aided analysis of AD and MCI. Moreover, we investigated the effectiveness of a diagnosis framework to identified stable mild cognitive impairment (sMCI) from convertible mild cognitive impairment (cMCI) by utilizing the effective features acquired form functional network of three frequency bands [slow-5 (0.01–0.027 Hz), slow-4 (0.027–0.08 Hz) and full-band (0.01–0.08 Hz)] at resting state. Graphics theory was implemented and modular -LASSO feature selection method was applied to searches the modular structure of function networks via signed spectral clustering technique. After this we selects the discriminative functional biomarkers via a modularity-induced group LASSO technique, followed by Extreme Learning Machine (ELM) for diagnosis classification of AD.

Results: The classification results obtained showed that the modular-LASSO features selection at slow-5 and all band have higher classification accuracy as compared to other frequency band and support to identify functional connectivity and discriminative brain regions related to AD.

Conclusions: The classification results obtained showed that the modular-LASSO features selection at slow-5 and all band have higher classification accuracy as compared to other frequency band and support to identify functional connectivity and discriminative brain regions related to AD.
Aims: Huntington's disease (HD) is associated with expansion of CAG more than 38 repeats in 4 chromosome. Intermediate alleles (more 30, but less than 38) are still undefined in phenomenology.

Methods: Case report of clinical manifestation in patient with intermediate allele length of Htt gene.

Results: The patient M., 47, suffered from low mood, hands' tremor, awkwardness, tearfulness and apathy from 2018. The patient's working status – medical nurse. She has no familial history of HD. In neurological status: slight saccade velocity slowing, high latency in speed of saccade initiation, Luria test – less than 4 repeats without hints, slight intentional and kinetic tremor, 2 deviation in tandem walking. While walking had some kind of dystonic pattern in neck with elevation of the right shoulder girdle. No choreic movements were found. She underwent genetic testing because of eye movement abnormalities that revealed 34 CAG-repeats. This result was explained to M. as a genetic 'grey area'. The patient's UHDRS score - 11, MOCA (26), SDMT (39), TMT-A (66 ss), TMT-B (100 ss), Stroop test for reading (98), naming (80), interference (40). According PBA-S main psycho-emotional problems were depression, anxiety, apathy, obsessive-compulsive disorders. Functionally the patient was healthy. After treatment with sertraline 50 mg, she felt substantial relief with Beck's depression scale score – 15.

Conclusions: In European ancestry populations, 1:5372 individuals from general population has intermediate allele expansion which result in the disease range during intergenerational transmission. This patient was recommended to follow-up and have genetic counselling for the family with further decision about the treatment and prognosis.
MIR-103A-3P/AGFG1 AXIS CONTRIBUTES TO PD PATHOGENESIS BY REGULATING ER STRESS AND APOPTOSIS.

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Aims: This study showed that microR-103a-3p level was decreased in substantial nigra of PD post-mortem and plasma of PD patients. AGFG1 was found to be a target of miR-103a-3p and this study showed that AGFG1 was highly expressed in substantial nigra of post-mortem of PD compared with healthy control. The current study focuses on exploring the underlying mechanisms.

Methods: The SH-SY5Y were transfected with miR-130a-3p mimics or miR-130a-3p inhibitor and AGFG1 overexpression or AGFG1 knockdown plasmid. Luciferase reporter assay was used to test the relation of miR-130a-3p and AGFG1.

Results: Overexpression of AGFG1 enhanced the TG-induced ER stress, shown from increased p-EIF2α, CHOP, GADD34 and CC3 in the AGFG1 overexpression plasmid transfected groups compared with control groups. Knockdown of AGFG1 attenuated the TG-induced ER stress in vitro. MiR-103a-3p mimics AGFG1 knockdown effect in response to ER stress that the increasing of p-EIF2α, CHOP, GADD34 and CC3 were attenuated in the miR-103a-3p mimic transfected groups. Inhibition of miR-103a-3p has the opposite effect to ER stress. Knockdown of AGFG1 can rescue the loss-of function in miR-103a-3p inhibitor induced ER stress with the p-EIF2α, CHOP, GADD34 and cleaved caspase-3 remaining unchanged in the miR-103a-3p inhibitor and AGFG1 knockdown plasmid co-transfected groups compared with control groups and less than the miR-103a-3p inhibitor transfected groups.

Conclusions: Our data revealed that miRNA-103a-3p functional involved in ER stress response pathway and protects against ER-stress induced cell death by targeting and suppressing AGFG1 and miR-103a-3p-AGFG1 regulatory pathway can be applied as therapeutic targets for the treatment of PD in the future.
Aims: The central nervous system (CNS) is surrounded and protected by three membranes called meninges. The development of the cerebral cortex and cerebellum requires primitive meninges. They produce trophic chemicals and extracellular matrix components that aid in the maintenance of homeostasis in the CNS and the healing of the blood-brain barrier following adult damage. Our goal was to compare the molecular and cellular features of fibroblasts from adult human donors' meninges to those of fibroblasts from the same donors' skin in order to identify the main aspects of this non-neuronal cell that is equally involved in CNS activities.

Methods: Meningeal fibroblasts (MFs) and skin fibroblasts (SFs) were isolated from brain donors. A qualitative analysis was performed starting from day 0 of culture. Different markers, Fibronectin, Serpinh1, Beta-III-Tubulin, and Nestin were analyzed by immunofluorescence. We performed the differentiation protocol in neuronal fate and also a whole transcriptome analysis.

Results: In cell culture, MFs and SFs have significantly varied appearances depending on the tissue function. Only the nestin protein was expressed differently in MFs and SFs. Transcriptome analysis revealed significant differences, particularly in cAMP metabolism and the cellular response to forskolin, a minor molecule used in many protocols for fibroblast to neuron transdifferentiation.

Conclusions: We may conclude that MFs and SFs are unique in terms of cell appearance and transcriptome profile. The upregulation of the pathway of forskolin could be the key for a possible replacement in situ for neuronal cells, without the pluripotent stage.
Aims: Develop a new model based on directly reprogrammed fibroblast into medium spiny neurons of striatum and mouse cortical neurons in order to study synaptic dysfunction in HD in vitro model

Methods: We utilized a protocol that was previously published (Richter et al., 2015) with some adjustments in order to obtain a homogeneous medium spiny neurons (MSN) population. After the end of reprogramming procedure, mouse cortical neurons from WT and YAC128 HD mice were inoculated. 14-17 days in co-culture cells were fixed and dendritic spine morphology, as well as synaptic marker level, were assessed.

Results: In the optimized protocol, >90% of the cells were stained for the striatal marker GABA and the MSN protein marker DARPP-32, in contrast to the original protocol, where only 70% of the cells were stained for GABA, and 60% were stained for DARPP-32. Reprogrammed MSNs are capable to form synapses with primary cortical neurons in co-culture with the formation of dendritic spines on the dendritic tree of MSNs. In HD co-cultures the total number of dendritic spines is significantly lower than in WT co-culture. Moreover, in HD co-cultures the was a significant decrease in mushroom spine numbers which are considered stable, functionally active synapses. The PSD95 and synapsin levels divided on DARPP-32 level were also decreased in HD co-cultures compare to WT cultures.

Conclusions: The developed model might be useful in the study of synaptic dysfunction in HD and also as a tool for screening therapeutic agents to correct observed impairments The study was support by RSF grant #22-25-00340
THE INTERACTION OF PARKIN AND TAF15 IN WHICH NEURONAL DEFECT WAS INDUCED

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Aims: Our study proposes a novel regulator for the protection of TAF15-induced proteinopathy and adds to our understanding of correlation between TAF15 and Parkin.

Methods: A. Drosophila stocks. All stock flies were raised at 25 °C on standard food. Crosses were performed using a standard procedure and all progeny were raised at 29 °C. B. Western blot analysis. Protein extracts for western blot analysis were prepared by homogenizing ten 14-day-old male fly heads. The total protein extracts (10 µg) were separated using a 4~12% gradient SDS-PAGE gel and transferred to PVDF membranes (Millipore). C. Immunohistochemistry. D. In vivo ubiquitination assay. E Life span and climbing assay.

Results: 1. Parkin is a novel binding partner and regulator for a TAF15-induced proteinopathy 2. Parkin regulates the protein level of TAF15 by ubiquitination

Conclusions: It was to identify a novel regulator for protection from neurotoxicity and to further understand the protective mechanism against a TAF15-induced proteinopathy, using genetic and molecular experimental approaches in Drosophila. Parkin overexpression during aging has been shown to decrease proteotoxicity. Our study provides in vivo evidence supporting the use of parkin for neuroprotection in TAF15-induced proteinopathy and new insights into the pathogenic mechanisms underlying TAF15-induced ALS. Finally, because the pathogenesis of TAF15-induced proteinopathy involves cytosolic aggregation of toxic proteins, it is important to have a mechanism that mediates protein degradation and prevents aggregate formation.
RETINOIC ACID ALLEVIATES INCREASE IN INTRACELLULAR CALCIUM CONCENTRATION AND THE DECREASE IN CALCIUM BINDING PROTEINS IN NEURONAL INJURY

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Aims: Ischemic stroke is the most common stroke and is caused by blood vessel occlusion. Retinoic acid is derived from vitamin A as a main product. It exerts various effects, such as anti-oxidant, anti-inflammation, and anti-apoptosis. Calcium regulates many biological processes including neurotransmitter release and ion channel permeability. Calcium binding proteins are involved in calcium cell signaling by binding to calcium ion. Parvalbumin and hippocalcin are calcium binding proteins and highly expressed in central nervous system. This study aimed to investigate whether retinoic acid has neuroprotective effect by regulating parvalbumin and hippocalcin expressions in cerebral ischemic injury and glutamate toxicity-induced neuronal cell damage.

Methods: Cerebral ischemia was induced by middle cerebral artery occlusion (MCAO) and retinoic acid (5 mg/kg) or vehicle was intraperitoneally injected for four days before MCAO. Neurobehavioral tests were performed 24 h after MCAO and brain was isolated.

Results: Retinoic acid alleviated MCAO-induced neurological deficits and histopathological changes. Expressions of parvalbumin and hippocalcin levels were decreased by MCAO, whereas retinoic acid treatment prevented these decreases. Moreover, glutamate toxicity induced cell death and increased intracellular calcium concentration in culture hippocampal cell and retinoic acid prevented these glutamate exposure-induced changes. Retinoic acid also mitigated glutamate exposure induced decrease of parvalbumin and hippocalcin expressions.

Conclusions: These results manifested that retinoic acid exerts neuroprotective effect by maintaining parvalbumin and hippocalcin expressions and regulating intracellular calcium concentration against cerebral ischemic injury and glutamate toxicity. This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST)(NRF-2021R1F1A105878711).
BAC TRANSGENIC SCA3 MOUSE MODEL EXHIBITS PROGRESSIVE DISEASE-LIKE PHENOTYPES AND REVEALS SHARED AND DISTINCT TRANSCRIPTOMIC SIGNATURES IN TRIPLET REPEAT DISORDERS

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Aims: Spinocerebellar ataxia type 3 (SCA3) is the most common dominantly inherited ataxia, and clinically it is characterized by progressive motor deficits and selective degeneration of neurons in the cerebellum, brainstem, and spinal cord. SCA3 is caused by CAG repeat expansion (>45) in the exon-10 of ataxin-3 (ATXN3). Currently, the pathogenic mechanisms of SCA3 remains elusive and there are no effective therapies.

Methods: To overcome the limitations of current SCA3 mouse models (i.e. relatively mild disease phenotypes), we aimed to develop a human genomic BAC transgenic mouse model of SCA3 (BAC-SCA3) with human ATXN3 cognate genomic context and an insertion of a long uninterrupted CAG repeat (>100 CAGs) in exon-10.

Results: We obtained two transgenic lines with multiple copies of the BAC transgenes. Detailed phenotypic studies revealed a BAC-SCA3 line mice exhibiting progressive adult-onset weight loss, multiple motor behavioral deficits, and brain atrophy. Neuropathologically, BAC-SCA3 mice showed ATXN3 immunoreactive aggregates in multiple brain regions, including cerebellum, cortex, hippocampus and brainstem, which also showed robust reactive gliosis. RNA-seq analyses of cerebellum, cortex and striatum of BAC-SCA3 mice at 10-month of age revealed between 1900 to 3000 significantly differentially expressed genes. Comparing the transcriptomes of BAC-SCA3 and those of Huntington’s Disease allelic series knockin mice revealed both shared and disease-specific gene signatures in these brain regions.

Conclusions: Together, our study provides a robust new human genomic SCA3 mouse model for mechanistic and preclinical study, and provides new insights on the converging and diverging pathogenic pathways among CAG triplet repeat disorders.
Aims: To evaluate the effect of bexarotene on protein expression changes associated with oligodendrocyte maturation in cortex and hippocampus in 3xTg-AD animals.

Methods: Triple transgenic animals for Alzheimer Disease (3xTg-AD) were treated with bexarotene (100mg / kg / day for 30 days). Subsequently, immunofluorescence analysis was performed by confocal microscopy of sections of brains treated with vehicle and bexarotene; and vehicle-treated controls (WT) for markers of oligodendrocyte maturation in the cortex and hippocampus regions.

Results: Bexarotene increased oligodendrocyte precursor cells in very old 3xTg-AD mice. In addition, there is a greater number of immature oligodendrocytes in the hippocampus and cortex, which co-localized with mitotic markers and the recovery of myelinating mature cells.

Conclusions: Bexarotene, an RXR receptor agonist, is a pharmacological candidate for the treatment of Alzheimer's disease as it is involved in the proliferation and maturation of oligodendrocyte precursor cells until myelinating oligodendrocytes that achieve the remyelination process.
DISCOVERY OF NOVEL SPHINGOSINE-1-PHOSPHATE-1 (S1P1) RECEPTOR AGONISTS FOR THE TREATMENT OF DEMYELINATING DISEASES

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Aims: The sphingosine-1-phosphate-1 (S1P1) receptor agonists have great potential to treat demyelinating diseases, such as multiple sclerosis (MS), because they can inhibit lymphocyte egress through receptor internalization. We designed and synthesized azetidine, pyrrolidine, and piperidine derivatives to discover a novel S1P1 agonist for the treatment of demyelinating diseases.

Methods: To evaluate functionally antagonistic S1P1 receptor agonist activities of the synthesized compounds, the compounds were assessed of their abilities to recruit β-arrestin to the S1P1 receptor and internalize the S1P1 receptor from the cell surface. Further evaluations of in vitro drug-like properties, pharmacokinetic studies, and cardiotoxicity of the compounds were conducted to determine a lead compound. Finally, the selected compound was subjected to peripheral lymphocyte count (PLC) assay and experimental autoimmune encephalitis (EAE) mouse model for the evaluation of its in vivo potency.

Results: Azetidine derivatives were determined to have excellent in vitro efficacy and drug-like properties. Among them, compound 21I was found to have superior drug-like properties as well as excellent in vitro efficacies (EC50 = 7.03 nM in β-arrestin recruitment; EC50 = 11.8 nM in internalization). We also confirmed that 21I effectively inhibited lymphocyte egress in the peripheral lymphocyte count (PLC) test and significantly improved the clinical score in the experimental autoimmune encephalitis (EAE) MS mouse model.

Conclusions: Consequently, such results suggest that 21I is a novel S1P1 receptor agonist for demyelinating diseases such as MS.
LOSS OF NPC1 ENHANCES PHAGOCYTIC UPTAKE AND IMPAIRS LIPID TRAFFICKING IN MICROGLIA

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Aims: Niemann-Pick type C disease is a rare neurodegenerative disorder mainly caused by mutations in NPC1, resulting in abnormal late endosomal/lysosomal lipid storage. Although microgliosis is a prominent pathological feature, direct consequences of NPC1 loss on microglial function remain not fully characterized.

Methods: To investigate pathological hallmarks of NPC in myeloid cells, we analyzed microglia from mouse models lacking NPC1 in the whole body or only in the myeloid cells and characterized defects of blood-derived macrophages from NPC patients. We performed immunohistological and proteomic analysis, combined with ex vivo and in vitro assays and electron microscopy to investigate phagocytic activity, lysosomal function and lipid trafficking of murine microglia/human macrophages upon NPC1 deficiency.

Results: We discovered a cell autonomous role of NPC1 in microglia underscored by pathological proteomic signatures and lipid trafficking defects. Loss of NPC1 triggers enhanced phagocytic uptake and impaired myelin turnover in microglia that precede neuronal death. NPC1-deficient microglia feature an accumulation of multivesicular bodies and impaired trafficking of lipids to lysosomes while lysosomal proteolytic function remains preserved. Molecular and functional defects were also detected in blood-derived macrophages of NPC patients.

Conclusions: Our study underscores an essential cell autonomous role for NPC1 in myeloid cells and implies microglial therapeutic potential. Furthermore, NPC patient macrophages recapitulate many of the key molecular and phenotypic features of murine NPC1-deficient microglia and thus represent a valuable tool for disease monitoring.
LOSS OF PROGRANULIN (PGRN) INFLUENCES THE INNATE AND ADAPTIVE IMMUNITY

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Aims: Progranulin (PGRN) is a secreted glycoprotein whose reduced expression is linked to several neurodegenerative diseases such as frontotemporal dementia (FTD), Alzheimer’s disease (AD), and Parkinson’s disease (PD). PGRN is expressed in neurons, brain-resident microglia, and peripheral immune cells. Although its specific function is still unclear, several studies have linked it with lysosomal functions and immune system regulation. Here, we have explored the consequences of PGRN deficiency in the central and peripheral immune systems.

Methods: For that, we used 19-24 months Grn-deficient mice and Grn-sufficient controls and assessed the immune phenotype in the brain and in the periphery of both male and female mice.

Results: Male Grn⁻/- mice exhibited a lower abundance of microglial cells, but with higher MHC-II expression, an increase in CD44 expression in monocytes, an increase in CD8+ T cells, and a decrease in CD4+ frequency of T cells in the brain. In the peripheral blood, we observed an increase in CD44 on CD8+ T cells. In contrast, female Grn⁻/- mice showed a decrease in microglia abundance with no changes in MHC-II expression and a reduction in CD4+ frequency of T cells in the brain. Additionally, we found an increase in Ly-6C-hi monocyte frequency and a decrease in CD44 expression in CD8+ and CD4+ T cells in the periphery.

Conclusions: Our data suggest that PGRN regulates the peripheral and the central immune system in a sex-specific manner; thus, understanding its associated mechanisms will pave the way for developing new strategies to modulate neuroinflammation in the context of neurodegenerative diseases.
NEW INSIGHTS INTO NIEMANN-PICK TYPE A DISEASE: UNRAVELLING THE MOLECULAR MECHANISMS LINKING SPHINGOMYELIN ACCUMULATION AND CELL DAMAGE

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Aims: Niemann-Pick Type A disease (NPA) is Lysosomal Storage Disorder caused by mutations in the gene coding for the acid sphingomyelinase, which imply the accumulation of its uncatabolized substrate sphingomyelin (SM). Since the molecular mechanism by which SM accumulation leads to cell death in NPA disease is still unknown, we developed and characterized a new in vitro model able to accumulate amounts of SM comparable to those observed in the more compromised NPA organs.

Methods: Fibroblasts derived from an NPA patient were loaded for 4 weeks with 50 µM SM and then characterized for the lipid composition and biochemical markers related to lysosomal function and cell damage.

Results: SM-loaded NPA fibroblasts accumulate SM, but also phospholipids, complex glycosphingolipids and cholesterol. Moreover, they present an increased nuclear translocation of the transcription factor EB (TFEB), together with an augmented lysosomal biogenesis and exocytosis, which enhances the activity of plasma membrane (PM) associated glycohydrolases. In addition, SM-loaded NPA fibroblasts are characterized by the activation of apoptosis and autophagy pathways. We confirmed these data, on a knock-out mouse model of NPA (ASMKO), where we discovered brains characterized by an increased TFEB nuclear translocation, responsible for the enhanced fusion of lysosomes with the PM, as well as by the activation of apoptosis and autophagy.

Conclusions: Taken together, our findings suggest that SM accumulation triggers lysosomal biogenesis and the fusion of lysosomes with the PM. This could lead to an aberrant PM catabolism of glycosphingolipids, probably responsible for the activation of cell damage pathways.
Aims: The glycogen storage disease type II (GSD II, Pompe disease) is a rare, multisystemic disease with variable rates of disease progression. It is caused by mutations in the acid α-glucosidase (GAA) gene, resulting in excessive accumulation of glycogen in different tissues, including the CNS. Here, we screened the 6neo mice, a mouse model of GAA deficiency, for behavioral deficits.

Methods: Homozygous 6neo mice of three different age groups were tested in the open field test, wire hanging test, grip strength test and beam walk test to measure general activity and anxiety, as well as muscle strength and motor deficits.

Results: In the grip strength test, 6neo mice present with deficits that could already be observed at the age of 8 weeks. In the open field test, wire hanging test and beam walk test 6neo mice demonstrate strong deficits, that can be observed at the age of 24 weeks.

Conclusions: Our behavioral analysis of 6neo mice suggests that GAA deficiency results in severe muscle weakness and motor deficits that can be objectively measured by different tests. This validates that 6neo mice represent an indispensable model for Pompe disease research, which can be effectively utilized for efficacy studies of potential new drug treatments.
Aims: Depression is a prevalent neuropsychiatric condition that increases the risk of developing neurodegenerative diseases. To investigate the mechanisms through which depression can increase this risk, we focused on the inflammatory changes that may occur. Using a corticosterone (CORT)-induced model of depression, we assessed inflammatory cytokines and innate immune markers systemically and in various brain regions across different durations of CORT administration.

Methods: CORT or vehicle was administered daily via subcutaneous injections to male Sprague-Dawley rats for 14, 21 or 28 days. Then, tissues from the liver, kidney, frontal cortex, hippocampus, striatum, thalamus and hypothalamus were processed for qPCR analysis.

Results: 14 days of CORT administration led to alterations of some of the markers assessed in the liver, including IL1b, with no alterations to other tissues assessed. At 21 days there were increases in various inflammatory cytokines and innate immune markers in the liver and the kidney. Additionally, the cytokines TNFa and IL1b in the striatum, IL1b in the thalamus and TNFa in the hypothalamus were all elevated. At 28 days, many of the altered markers at 21 days returned to baseline in the liver and kidney. However, TNFa and IL1b in the kidney and IL1b in the liver remained elevated. Additionally, TNFa was elevated in the frontal cortex and striatum following 28 days of CORT administration.

Conclusions: Although glucocorticoids are classically thought of as anti-inflammatory, these results suggest that chronically high levels of glucocorticoids may lead to dysregulation of immune mechanisms.
Aims: To investigate all cognitive domains in Parkinson’s Disease Dementia (PDD) patients using the tasks from the CDR System, a computerized cognitive battery.

Methods: Data from the CDR System database were used and attention, episodic and working memory measures were extracted in order to calculate composite scores relevant to the patient population and compared these to age-matched healthy volunteers corresponding measures. Data were analyzed using mixed model analyses of variance and effect sizes (ES) were calculated.

Results: The analysis revealed significant impairments in cognitive domains. Speed of attention is the most impaired, with an ES of - 5.2, corresponding to an average impairment of 655 milliseconds. Accuracy and fluctuation of attention were also impaired with an ES of - 0.99 and - 1.26 respectively. Speed of working memory (ES = - 3.04; equivalent to 1.6 second) and speed of Episodic memory (ES = - 1.83; equivalent to 1 second) are also strongly impaired and to a greater extent than their respective accuracy (Working Memory ES= - 0.73; Episodic memory= - 0.80).

Conclusions: The CDR system identified deficits in cognitive domains in PDD patients. Effect Sizes for most of these measures are of large magnitude. Overall, automated tests of cognitive function can identify patterns of impairment in PDD patients, some of which are not detected by traditional tests. The CDR System is a useful cognition tool, able to detect with precision cognitive domains affected in PDD patients and can identify drugs that may modulate these cognitive domains.
THERAPEUTIC BENEFITS OF RTMS IN MODERATE TO SEVERE ORGANIC DEPRESSIVE EPISODES

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Aims: Motivation: rTMS is the repeated administration of pulses which, depending on the parameters (intensity, frequency, duration) induce a change in the level of cortical excitability. Low frequency stimulation has an inhibitory effect, while high frequencies induce an increase in cortical excitability. Experimental studies support a modulation by magnetic stimulation of catecholamine metabolism.

Objectives: Explicit of the beneficial results of rTMS on a group of 30 patients diagnosed with depressive episodes of minimum moderate intensity after stroke. The parameters used varied depending on the individual motor threshold detected, using frequencies of the order of 20 Hz, applied in series of 400-1200 pulses per day, for 10 days.

Methods: rTMS in depression has as a pathophysiological basis the existence of a metabolic asymmetry between the two hemispheres. Functional imaging showed a decrease in metabolism in the dorsolateral prefrontal cortex. rTMS with a high frequency increases the rate of metabolism, restores this imbalance, having a beneficial effect on depression.

Results: The change in the level of excitability is mainly reflected in the cortex underlying the stimulation, but, due to the cortico-cortical connections, distant changes can also be generated. rTMS applied with the same parameters, several consecutive days on patients with post-stroke depressive organic affective disorder, through a cumulative effect, induces effects that persist for a long time, maintaining antidepressant treatment.

Conclusions: The effect of magnetic stimulation in depression, appreciated by the Hamilton scale, was favorable, the improvement persisting for several months. High frequency magnetic stimulation also had results in aphasia recovery.
Aims: This paper aims to highlight a group of 30 patients admitted to the psychiatric service, who experienced an ethanol withdrawal complicated by delirium tremens and who developed ischemic stroke on average 72 hours after the onset of the first manifestations of withdrawal, the background being that of high blood pressure, it is not enough to reduce high values exclusively with benzodiazepines.

Methods: Psychiatric evaluation and crossing a complicated service with delirium tremens, correct dosage of psychoactive medication due to impaired metabolism in alcohol consumption disorder; administration of new generation anticonvulsants as the first line of selection.

Results: Alcohol disorder involves subclasses, from mild to severe, ranging from episodic excessive alcohol consumption (min 0.8 g / dl in a short period of time), to early weaning, late weaning, complicated weaning with DT, complicated withdrawal with epileptic seizures. The risks of a patient experiencing delirium tremens occurring with an ischemic or haemorrhagic stroke depend on: hydroelectrolytic imbalances, malignant cardiac arrhythmias, untreated hypertension (and treated exclusively with benzodiazepines), prolonged seizures, craniocebral trauma.

Conclusions: Liver failure can lead to hepatic encephalopathy, which involves altered consciousness, accumulation of ammonia toxins, altered liver function and the onset of transient strokes, which can occur within the first 72 hours and can lead to ischemic or haemorrhagic accidents due to hypertension, psychomotor agitation, visual hallucinations and changes in the field of consciousness.
Aims: The aim of this investigation is to evaluate the effect of masupirdine on agitation symptoms using the 12-item neuropsychiatric inventory (NPI-12) scale in Alzheimer's dementia (AD) patients.

Methods: Masupirdine was evaluated in a multicenter, randomized, double-blind, parallel group, 26-week, placebo-controlled proof of concept phase-2 clinical trial in subjects with moderate AD (NCT02580305). Subgroup analyses were carried out on the agitation/aggression domain of the NPI-12 scale. Analyses were based on the independent patient subgroups with baseline symptoms. A mixed-effects model for repeated measures was used to determine the effect of masupirdine on agitation/aggression symptoms in modified intention to treat population.

Results: A statistically significant (p<0.01) reduction in agitation/aggression scores was observed in patients treated with masupirdine (50 & 100 mg) at Week 13. The effect was sustained till the end of treatment (Week 26). Effect size (Cohen's d) observed in the masupirdine 50 mg treatment arm at the end of 26 weeks was 0.66, suggesting clinically meaningful effect.

Conclusions: Further exploration is warranted to confirm the beneficial effects of masupirdine on agitation. Currently, a phase-3 study to evaluate the effects of masupirdine on agitation in patients with dementia of the Alzheimer’s type is in progress.
Aims: Psychotic symptoms in Parkinson's disease are common and include hallucinations, illusions and delusions. Psychosis is a major cause of disability and is one of the most distressing symptoms for both patient and caregiver. The management of psychotic symptoms in Parkinson's disease is often complex and challenging and involves determining the degree and severity of psychotic symptoms and whether interventions are required. In this abstract, current evidence-based treatment options are reviewed.

Methods: Review of the current literature on Parkinson's disease psychosis including randomized, double blind, placebo controlled trials

Results: Clozapine and Pimavanserin have proven efficacy in the treatment of Parkinson's disease psychosis without impairing motor function. While quetiapine is frequently used, its evidence is not supported by double blind, randomized placebo controlled trials.

Conclusions: Clozapine and pimavanserin should be considered first line treatment of Parkinson's disease psychosis at this time. Although quetiapine did not show efficacy, it is often used to treat Parkinson's disease psychosis as it does not worsen motor function and lacks the blood monitoring requirement of clozapine.
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A RANDOMIZED CLINICAL TRIAL TO EVALUATE THE EFFECTS OF SAFINAMIDE ON APATHETIC NON-DEMENTED PARKINSON'S DISEASE PATIENTS

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Aims: To explore the effects of Safinamide for the treatment of apathy in patients with Parkinson's disease without dementia.

Methods: A prospective, 24-week, two-site, randomized, double-blind, placebo-controlled, parallel-group exploratory study (EudraCT 2017-003254-17) in non-demented PD subjects on stable dopaminergic therapy randomized 1:1 to adjunct safinamide (50mg/day for 2 weeks and 100mg/day for 22 weeks) or placebo. Primary endpoint: Mean change from baseline to week 24 on the Apathy Scale (AS) total score. Secondary outcomes: Changes in cognition, activities of daily living, motor scores, and impression of change, safety and tolerability measures.

Results: Early termination due to Covid-19 pandemic precluded inclusion of planned sample size (N=36). 30 participants (active treatment=15; placebo=15) were enrolled and included in the intention-to-treat analysis. Change in AS showed a trend to significance [p = 0.059] mediated by a more marked decrease on AS score in the safinamide group (-7.5 ± 6.9) than the placebo group (-2.8 ± 5.7). In the active treatment group no differences existed between baseline and 12-week AS score while a significant difference was found between 24-week and 12-week [p = 0.001]. In the placebo group, no significant differences were found. No significant or trend changes were found for any of the secondary outcomes. Adverse events were few and only mild in both treatment groups.

Conclusions: Safinamide treatment resulted in a trend to significant reduction of apathy at 24 weeks (primary objective) and a significant but delayed improvement between 12 and 24-weeks. Safinamide was well tolerated and resulted in a significant improvement of apathy in PD.
Aims: The aim of this study was to analyze the association between cerebrospinal fluid (CSF) Alzheimer’s Disease (AD) biomarkers and depression or suicidal ideation (SI) in patients with Mild Cognitive Impairment (MCI).

Methods: 59 patients age > 50 years old with criteria for of MCI positive (MCI-AD) (n=22) and negative (MCI-Non AD) (n=24) AD and healthy controls (HC) (n=13) were included in the study. Depression was assessed using the Beck Depression Inventory I (BDI-I) and the Geriatric Depression Scale (GDS-30), and SI with the GDS-SI factor. Additionally, Phosphorylated-tau (P-Tau), total-tau (T-Tau) and amyloid beta 42 (Aβ42) were measured in CSF.

Results: We found significant differences in the BDI and SI scores between the three groups. Exactly, the MCI-AD group presented lower depression scores than the MCI-Non AD group, but we did not found differences between the MCI-AD or MCI-Non AD groups and the control group. In the MCI-AD, higher CSF Aβ levels were associated to higher depression, whereas lower P-tau and T-tau levels were related to higher SI. However, in the MCI-Non AD and HC groups no relationship between AD biomarkers and depression or SI was observed.

Conclusions: Our results indicate that MCI-AD patients with lower AD pathology show higher risk of depression and SI. This result underline the significance of taking in account depression and SI in early stages of AD, and the potential value of AD biomarkers in the early detection of these symptoms.
EMOTION RECOGNITION AND CORTISOL LEVELS RELATIONSHIP IN ALZHEIMER’S DISEASE

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Aims: The objective of this work was to study the relationship between complex emotions recognition and cortisol levels in participants cognitively unimpaired, with mild cognitive impairment (MCI) and dementia due to Alzheimer Disease (AD).

Methods: Complex emotions recognition were evaluated with Reading the Mind in the Eyes Test (RMET) and plasma cortisol levels were determined in the morning in the following groups: mild dementia due to AD (MD-AD, n=20), mild dementia non-AD (MD non-AD, n=13), MCI due to AD (MCI-AD, n=25), MCI non-AD (n=34) and healthy controls (HC, n=16).

Results: Significant lower positive emotions recognition in the MCI non-AD than the healthy group were found (p = 0.02). Additionally, significant lower emotions recognition were obtained between groups with MD (AD and non-AD) compared with the healthy group (p < 0.01). We observed significant differences in cortisol and all RMET scores between the MCI and MD groups (p < 0.01). Furthermore, multivariate regression analyses showed an association between RMET scores and higher cortisol levels, including sex, age and educational level as covariates (p < 0.01). Finally, we found correlation between RMET total scores and higher levels of cortisol but only in MD groups (p = 0.01).

Conclusions: These outcomes suggest that detection of positive emotion dysfunction could help to identify the MCI-non AD patients. In addition, global emotion recognition implies higher cognitive impairment at dementia level. Hypercortisolemia may imply failure in positive emotion recognition in the total group and worse global emotion recognition in the AD and non-AD groups.
AMYLOID BURDEN EVALUATION USING F-18 FLORBETABEN PET IN PATIENTS WITH ALZHEIMER’S DEMENTIA, MILD COGNITIVE IMPAIRMENT AND SUBJECTIVE COGNITIVE DECLINE

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Aims: The aim of the present study is to evaluate the performance characteristics of florbetaben F18 positron emission tomography (PET) in patients with Alzheimer's disease (AD), mild cognitive impairment (MCI), subjective cognitive decline (SCD), and healthy control (HCs).

Methods: Three hundred nineteen participants (146 AD, 100 MCI, 50 SCD and 23 HCs) were recruited and underwent a F-18 florbetaben PET scan. Amyloid burden was assessed visually and quantitatively, and was classified as positive or negative. The amyloid PET images were reviewed by two nuclear physicians according to the scoring system and classified as positive or negative. We simultaneously assessed quantitative analysis using a threshold for amyloid positivity of 1.26 for the mean cortical standard uptake value ratio.

Results: Florbetaben PET was rated visually amyloid positive in 88% of AD patients, 40% of MCI patients, and 26% of SCD and 4% HCs. Seventy-eight percent (114/146) of AD patients, 39% (39/100) of MCI patients, and 30% (15/50) of SCD and 9% (2/23) HCs were classified as amyloid positive using a quantitative threshold (mcSUVR > 1.26). Florbetaben cortical retention was highest in subjects with AD (mcSUVR=1.35 ± 0.15) and lowest in cognitively normal subjects. Amyloid positivity and mean cortical amyloid burden were associated with age and apolipoprotein E ε4 carrier status.

Conclusions: The current results are consistent with expected rates of amyloid positivity among individuals with clinical diagnoses of AD and MCI, and indicate the potential value of florbetaben F18 PET as an adjunct to clinical diagnosis and amyloid burden effect of progression of Alzheimer's disease spectrum.
Aims: Anxiety early in Parkinson's disease may stem from dysregulation of striatal "patch" or "striosome" compartments as these are enriched in PD-linked LRRK2, receive abundant dopamine from fragile ventral tier SNc, and are embedded in more limbic circuitry relative to surrounding striatal matrix. We sought to test whether striosomal territories mediate anxiety behavior. Secondly, we sought to investigate circuit anatomy particular to striosome manipulation in our mouse model.

Methods: We tested behavior using a SepW1-NP67 striosome-Cre mouse to achieve genetically targeted striosomal ablation, calcium-imaging, and chemogenetics in the context of a modified Light/Dark box test. We tested circuitry using synaptic density quantification in striatal output nuclei with a cre-dependent synaptophysin infused GFP.

Results: We find that major measures of anxiety (stay time in light/dark) are unchanged, but that locomotor speed reflects the unlearned, external valence differential present in the Light/Dark box. We find that striosomes gate valence-oriented speed selection. We observe denser direct pathway innervation in the internal pallidum, with more diffuse indirect pathway synapse formation.

Conclusions: We provide a more intricate understanding of striosomes regulation of anxiogenic locomotor response, as well as circuit architecture that leads to complex striosome-related behavior. These data identify striosomal dysregulation as a potential source of impaired valence/velocity integration.
DIFFERENTIAL EFFECTS OF AMYLOID-B AND TAU NEUROPATHOLOGY ON COGNITIVE AND EMOTIONAL SYMPTOMS IN NOVEL ALZHEIMER’S DISEASE TRANSGENIC MICE

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Aims: Amyloid plaques and neurofibrillary tangles containing amyloid-β (Aβ) peptides and hyperphosphorylated tau protein, respectively, are the neuropathological hallmarks of Alzheimer’s disease (AD). However, how concurrent Aβ and tau pathologies synergize to disrupt specific brain circuits during AD progression is not fully understood.

Methods: Here, we characterize novel double transgenic mice expressing mutant human amyloid precursor protein (APP) and microtubule-associated protein tau (Tau) in excitatory neurons, to evaluate age, sex, and pathological effects on brain circuitry and behavior.

Results: We detected transgene effects, but not gender differences, on neuropathological progression and cognitive and emotional deficits. Interestingly, tau phosphorylation and aggregation were increased in the hippocampus and amygdala of double APP/Tau mice compared to single mutant Tau mice, whereas Aβ pathology was not affected by mutant Tau expression. Moreover, adult APP and APP/Tau, but not Tau mice, exhibit anxious behavior and impaired fear memory extinction associated with increased Aβ, but not tau pathology, in limbic regions. By using tissue clearing and imaging, we show that memory deficits coincide with impaired activation of excitatory neurons, synaptic accumulation of tau and reduced presynaptic proteins in the hippocampus of APP/Tau mice.

Conclusions: Our results indicate that the progression and interaction of Aβ and tau pathologies in specific brain circuits exacerbates synaptic pathology and memory and emotional symptoms in AD.
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THE VIRTUAL DEMENTIA TOUR® ITALIAN VALIDATION. SUPPORTING EMPATHETIC DISPOSITION FOR A BETTER CAREGIVING

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Aims: Empathy towards a person with dementia improves the quality of their care. However, people with dementia are changed because of the disease and this change can make caregiving difficult. Being able to ‘walk in the shoes’ of a person with dementia fosters empathic attunement, and this is what the Virtual Dementia Tour® (VDT®) has been doing worldwide for 20 years involving caregivers (Beville 2002). Until now the VDT® has never been translated and validated in any other language other than English.

Methods: We introduce the Italian version of the VDT®, obtained with a forward and back-translation process, and we discuss its internal validity, main factor analysis, and reliability. The Italian validation involved 419 people (mean age = 44.8; ds = 14.2) who underwent an 8-minute experience in which they were asked to perform some daily activities while their abilities were altered by special devices simulating the symptoms of dementia. Before and after the tour they were asked to answer a series of questions aimed at understanding the needs and the challenges experienced by a person with dementia.

Results: The manual translation did not reveal any significant inconsistencies during the conversion and cultural adaptation. The Italian version of VDT® revealed a construct validity (2 main factors: empathy and autonomy) and the test-retest analysis highlighted how the tour had a positive influence on empathy.

Conclusions: The data also suggest, in the Italian version, the significant educational-experiential value of VDT® in supporting the introduction of a higher quality of care for people living with dementia.
POSTERS

IDENTIFYING FAMILIARITY AND KNOWLEDGE OF ADUCANUMAB IN CAREGIVERS OF HAWAI’I ALZHEIMER’S DISEASE PATIENTS

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Aims: Aducanumab, the first potential disease modifying treatment for AD, was granted accelerated approval by the FDA in June 2021. There is a paucity of data regarding the familiarity of aducanumab among patients with AD, their families, and caregivers. Identifying current perceptions within these populations is vital for proper management and counseling by health care providers.

Methods: A quality improvement telephone survey was administered to 339 caregivers of patients with AD who were seen at our institution within the last two years. The 10-minute phone survey included questions inquiring about current management and disease progression, knowledge of aducanumab, and demographic data. Bivariate analyses included nonparametric testing with an alpha < 0.05.

Results: Out of 339 eligible caregivers, 85 (25.1%) survey responses were collected. The majority (63.5%) of caregivers were unfamiliar with aducanumab. Familiarity was higher amongst caregivers who were spouses of the patient as opposed to others like children or relatives of patients (OR=5.21; 95%CI: 1.75-16.56; p=0.0016) and caregivers who had recently heard about AD in the news (OR=22.91; 95%CI: 4.93-218.10; p<0.001). Decreased familiarity was seen in caregivers who identified as Native Hawaiian or other Pacific Islander (NHPI; OR=0.24; 95%CI: 0.041-0.96; p=0.032).

Conclusions: Many caregivers of patients with AD in Hawai’i are not yet familiar with aducanumab, especially those caregivers who are NHPI or not spouses of their patients. Additional counseling with these caregivers may increase understanding of this treatment option and overall better management of patients with AD.
IMPROVING DEMENTIA CARE THROUGH DIGITAL CAREGIVER EMPOWERMENT: A PRAGMATIC REAL WORLD TRIAL

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Aims: The Ceresti Digital Caregiver Empowerment Program (DCEP) was deployed to family caregivers of Medicare Advantage patients with Alzheimer’s Disease and other dementias (ADOD). The primary objective of this pragmatic trial was to demonstrate that more knowledgeable, skilled and confident family caregivers can reduce unnecessary healthcare utilization. Primary and secondary objectives included the following: Patient Outcomes • Reduction in hospitalizations • Reduction in emergency department visits • Reduction in 30-day hospital re-admissions • Increase in medication adherence • Reduction in medical costs Caregiver outcomes • Improved mental health Population metrics • Reduction in risk trajectory

Methods: Patient outcomes were compared to outcomes from a propensity matched control group. The impact of the DCEP was determined by comparing the pre-index averages to outcomes from the 30-day post-index period (Figure 1).

Figure 1. Claims Analysis Methodology

Three (3) matched control patients were selected for every enrollee patient based on propensity matching on demographic, medical, utilization, and cost variables. Predictive Analytics
The DCEP was personalized for each caregiver/patient dyad using predictive models applied to claims data. Risk of hospitalization was determined for the most common preventable hospitalization conditions and caregiver education and support was prioritized based on patient risk.

Results: Intervention Results illustrated below:
Patient Outcomes
Table 1 summarizes statistically significant (p-values < 0.05) reductions in utilization for patients enrolled > 45 days, and enrolled high utilizers enrolled > 45 days. High utilizers are patients with at least one hospitalization or two ED visits in the prior 24 months.

The increase in drug costs suggests an increase in medication adherence that is borderline statistically significant.

Table 1

<table>
<thead>
<tr>
<th>Outcomes for Enrollees vs Control, more than 30 days post-index, to end of claims data</th>
<th>ALL ENROLLED (N=131, Eligibility = 7.24 mos)</th>
<th>ENROLLED HIGH UTILIZERS (N=62, Eligibility = 7.12 mos)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Relative Diff.</td>
<td>P-value</td>
</tr>
<tr>
<td>Inpatient Admissions per 1,000 Patients per year</td>
<td>-513</td>
<td>0.02</td>
</tr>
<tr>
<td>ED Visits per 1,000 Patients per year</td>
<td>-424</td>
<td>0.03</td>
</tr>
<tr>
<td>30 Day Readmissions Rate</td>
<td>-30%</td>
<td>0.02</td>
</tr>
<tr>
<td>Medical Costs (patient / mo)</td>
<td>-$666</td>
<td>0.003</td>
</tr>
<tr>
<td>Inpatient Costs (patient/mo)</td>
<td>-$491</td>
<td>0.003</td>
</tr>
<tr>
<td>Drug Costs (patient/mo)</td>
<td>$53</td>
<td>0.05</td>
</tr>
</tbody>
</table>
Conclusions: Empowering family caregivers of ADOD patients is effective in: (i) reducing unnecessary healthcare utilization (ii) increasing patient medication adherence (iii) reducing patient medical costs (iv) improving caregiver mental health. In addition, empowering caregivers changes the rate at which low-utilizer patients become high utilizers, thus reducing the risk trajectory of a population of ADOD patients.
Aims: Research has proposed that cognitive training (CT) can be used as an intervention to remediate age-related cognitive declines and dementia. The current study aimed to explore proximal transfer effects within 8 attention/processing speed and working memory tasks delivered through adaptive CT intervention.

Methods: 78 healthy older adults were assigned to either an adaptive CT intervention (n=39) or educational training control (ET; n=39) over the course of 12-weeks. The CT condition consisted of 40 hours of working memory and attention/processing speed training. The ET condition had participants watch 40 hours of educational videos. All participants underwent active or sham transcranial direct current stimulation (tDCS), although not a variable of interest. Repeated-measures ANCOVA assessed pre/post change in subtask scores between intervention groups, controlling for age, sex, education and tDCS group. Bonferroni-corrected α of p=0.00625 was set to correct for multiple comparisons.

Results: Repeated-measures ANCOVAs revealed significant timepoint*intervention interactions for all 8 subtasks (all p-values< 0.00625). Cohen’s f was calculated to assess relative strength. Participants in the CT intervention had higher scores on working memory post-intervention compared to ET (Cohen’s f=0.12-0.32). Participants in the CT intervention had significantly faster performance on reaction time tasks post-intervention compared to ET (Cohen’s f=0.19-0.54). No main effect of timepoint for the collapsed CT/ET groups were found for any of the 8 subtasks (all p-values>0.05).

Conclusions: Our findings suggest that CT interventions produce significant proximal transfer effects. Double Decision, a processing speed task, had the largest Cohen’s f=.54. Our data signifies that effect sizes were driven by improvements of the CT group.
Aims: The tDCS may augment cognitive training in aging. However, effects are inconsistent across studies. The baseline brain state inter-individual differences may play a role in this inconsistency of results. We utilized data from our previous study of bifrontal tDCS coupled with cognitive training (Šimko et al., 2021) to explore predictors of tDCS effects with a new insight from dynamic resting state functional connectivity [DFC].

Methods: Twenty-five healthy seniors (68.84 ± 4.65 years) participated in a double-blind randomized sham-controlled trial with a cross-over design. Before each tDCS session (20 minutes of 2mA) participants underwent a resting-state fMRI. The visual working memory task (vWMT) was performed prior to and immediately after each session. Using ICA, we identified 10 large-scale brain networks. DFC analysis was employed to reveal DFC states based on the temporal variability in rs-FC (Allen et al., 2018). We identified 4 DFC states and state occurrence was parameter of interest (Díez-Cirarda et al., 2018). Stepwise regression analysis was performed to find the best predictive variable for the magnitude of tDCS-induced improvements in vWMT accuracy.

Results: The occurrence of DFC state 4 was a significant predictor of tDCS-induced vWMT accuracy changes ($R^2 = 0.22$, $p = 0.035$). A high correlation between the frontoparietal and the language network characterized the state, probably representing readiness to top down control of language processing.

Conclusions: We show that the brain state as measured by the DFC plays a role in inter-individual differences in tDCS-induced cognitive aftereffects. The DFC analysis outperformed a regular SFC analysis for identifying tDCS responders.
POSTERS

EFFECTS OF NON-INVASIVE BRAIN STIMULATION IN ALZHEIMER’S DISEASE AND MILD COGNITIVE IMPAIRMENT? - RESULTS FROM META-ANALYSIS

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Aims: Alzheimer's disease (AD) and its preclinical stage, mild cognitive impairment (MCI), are critical issues confronting the aging society. Non-invasive brain stimulation (NIBS) techniques have the potential to be effective tools for improving cognitive functioning. The main objective of our meta-analysis was to update the status of the efficacy of repetitive Transcranial Magnetic Stimulation (rTMS) and Transcranial Direct Current Stimulation (tDCS) when applied in AD and MCI.

Methods: We conducted a systematic search on PubMed / Web of Science for blinded randomized control studies or studies with crossover design involving human subjects using rTMS, tDCS as a tool for cognitive enhancement in AD and MCI in May 2021. Risk of Bias (RoB) assessment was used to only include works with high methodological quality. Hedge’s g was used to determine effect size. We focused on the immediate and long-term effects (~1-month follow-up).

Results: Based on the PRISMA-statement and RoB analysis we included 16 rTMS and 16 tDCS studies into the meta-analysis. We found both, rTMS and tDCS to have significant immediate cognition-enhancing effect in AD and MCI with rTMS having moderate (g=0.57; 95% CI=0.4, 0.73) and tDCS small effect (g=42; 95% CI=0.13, 0.70). rTMS effect were moderate after at least 1 month of follow-up (g=0.82; 95%CI=0.2, 1.45; p<0.01). For tDCS, the long-term effects were statistically heterogeneous.

Conclusions: In conclusion, we found NEURO-AD™ system. High-frequency rTMS, and anodal tDCS over the left DLPFC as probably effective protocols in AD. Regarding NIBS in MCI, further research and replication of existing studies is required to identify optimal protocol.
Aims: Cognitive dysfunction is a common indicator of dementia which is associated with various chronic diseases. Dementia, along with chronic diseases, affects overall health. The specific aim in this study was to understand if hypertension influenced the overall effect of cognitive dysfunction on all-cause mortality.

Methods: The 1999-2002 National Health and Nutrition Examination Surveys with mortality data obtained through 2015 was a nationally representative population. African American adults aged 60 years or older were assessed for cognitive skills using Digit Symbol Substitution Test (DSST) and compared to other races. Analysis was performed using complex samples Cox regression to determine the relationship of cognitive dysfunction on mortality and how hypertension influences this.

Results: Percent with cognitive dysfunction was higher among African American females (57.7%) than males (42.3%). For all-cause mortality, the overall unadjusted hazard ratio (HR) of low cognitive function was 2.69 (95% confidence interval [CI], 1.55-4.68, p = 0.001). Adjusted HR was elevated, 1.96 (CI 1.13-3.40, p = 0.02), among individuals with hypertension and cognitive dysfunction but closer to 1.0 (1.05 CI 0.44-2.52, p = 0.91) among those with no hypertension and cognitive dysfunction, after controlling for medical (coronary heart disease) and demographic risk factors (age and sex) among African Americans. Similar patterns did not exist in Caucasian adults.

Conclusions: According to our research, cognitive dysfunction is associated with all-cause mortality more strongly in African Americans with hypertension than those without. Outcome disparities may be a result of exposure and impact of chronic stress, racial discrimination, and mental health distress.
POSTERS

SIMPLE VOICE BIOMARKER DURING SPONTANEOUS SPEECH FOR DETECTING MILD COGNITIVE IMPAIRMENT

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**Aims:** As the world's population is aging, dementia has become a major social issue. The early detection and intervention for cognitive decline are essential factors for solving this problem. Although the Montreal Cognitive Assessment (MoCA) score is used to assess mild cognitive impairment, it is challenging to use it for daily monitoring because it is an interview-style. This study investigated the possibility of predicting MoCA scores from speech, which is an easier biomarker to obtain.

**Methods:** We recorded the voices of 60 participants at two hospitals. The participants consisted of 25 males and 35 females with a mean age of 82.5±5.4 years. We used the Japanese version of MoCA. We recorded the conversation of the MoCA interview. To separate the interviewer from the participant, pin microphones were attached to them and each connected to different channels. We cut the audio data into 40 ms frames and calculated each frame's maximum sound pressure level to create a data point sequence, classifying them into two groups using Gaussian Mixture Modeling. The lower sound level group was labeled as silence, and another was labeled as speech. We compared the descriptive statistics of the distribution of continuous duration of silence and MoCA scores.

**Results:** The correlation coefficient between MoCA score and the mean of the distribution was $r=-0.37$ ($p=0.003$), interquartile range was $r=-0.34$ ($p=0.008$), and third quartile was $r=-0.30$ ($p=0.02$).
**Conclusions:** The result shows that these statistics and MoCA score have significant correlations, respectively. The possibility of predicting cognitive function level using a simple feature was shown.
LIFE-LOG BASED MILD COGNITIVE IMPAIRMENT PREDICTION USING DEEP LEARNING

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Aims: Conventional methods for diagnosing cognitive impairment such as MRI, PET, and SNSB, are difficult to access because of high economic and time costs. To overcome the problems, we present a deep learning algorithm for low-cost and early detection of cognitive impairment based on life-log data acquired from wearable healthcare devices. The proposed algorithm performs binary classification into the cognitive normal group or the cognitive impairment (Mild Cognitive Impairment, Dementia) group.

Methods: The life log data set consisted of information on 375 subjects, including 258 cognitive normal, 107 mild cognitive impairment, and 20 dementia, and was acquired for 63.0(-24.7) days for each subject. 35 days of sequential data were generated through the sliding window method. 5-fold cross validation was used to avoid overfitting to one test data.

Results: As a result of the classification model predicting the data selected by the filtering model, the confusion matrix is as follows:

<table>
<thead>
<tr>
<th></th>
<th>CI Truth</th>
<th>CN Truth</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI Pred</td>
<td>326 (45%)</td>
<td>279 (20%)</td>
</tr>
<tr>
<td>CN Pred</td>
<td>403 (55%)</td>
<td>1113 (80%)</td>
</tr>
</tbody>
</table>

Accuracy : 0.678 AUC : 0.696

Conclusions: Through life log data of about 35days, it was possible to diagnose subjects with 67% accuracy.
Aims: Dementia is relatively common in older adults. Its detection at the mild cognitive impairment (MCI) stage is essential for managing the symptoms. Vocal analysis is an easy, device-independent, and effective diagnostic technique. In this study, we investigated the detection of MCI on the basis of vocal analysis.

Methods: We collected voice data of the long vowel (/Ah/) of 58 participants from two hospitals. We employed the MoCA's Japanese version (MoCA-J) to screen the participants for MCI. The psychomotor functions were adversely influenced by cognitive decline. Therefore, we calculated three acoustic features—jitter, shimmer, and harmonics-to-noise ratio—from each item in the voice data. Further, each item in the voice data was split into three 0.5-s phonatory sections (i.e., beginning, middle, and end sections) of an utterance to evaluate symptomatic differences, and their three acoustic features were analyzed. The correlations between the acoustic features and the MoCA-J score were analyzed.

Results: The correlation coefficients between the three acoustic features were insignificant, as reflected by the MoCA-J score calculated from using the entire acoustic data. The jitter ($r = -0.297; p = 0.023$) and shimmer ($r = -0.273; p = 0.038$) at the beginning sections of the utterances exhibited significant correlation coefficients.

Conclusions: Thus, long vowel articulation can reflect the cognitive decline-influenced psychomotor functions. Analysis of the beginning sections of long vowel utterances may potentially help detect MCI.
DETECTION OF MILD COGNITIVE IMPAIRMENT USING DIFFERENCE OF VOICE FEATURES BETWEEN CONDITIONS

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¹The University of Tokyo, Department Of Bioengineering, Graduate School Of Engineering, Tokyo, Japan, ²PST Inc., Research And Development, Yokohama, Japan, ³Kanagawa University of Human Services, Graduate School Of Health Innovation, Kawasaki, Japan

Aims: Early detection of mild cognitive impairment (MCI) is a key technology for preventive medicine in cognitive disorders. A voice-based screening is a competitive candidate of screening tool in daily life for its lower cost and ease of use. We tried to build a prediction model to detect MCI using voice features recorded in conditions with and without a cognitive task.

Methods: We recorded participants’ voice when they just read numbers and when they did subtraction in their head. We acquired voice features in both conditions. We evaluated their cognitive impairment by Mini Mental State Examination (MMSE). The participants whose score of MMSE was equal to or lower than 27 were regarded as MCI (including dementia). We built two classifiers by extreme gradient boosting algorithm using two voice feature set: set A was acquired only from voice when they did subtraction in their head, and set B was acquired as difference of features between two recording conditions. The numbers of features were almost same (6506 in A and 6510 in B). Training set consisted of 199 participants and test set consisted of 20 participants independent from training set.

Results: As the result, the classifier using B achieved higher performance than one using A: area under curve of receiver operating characteristic curve for the classifier using A was 0.68 while that for the classifier using B was 0.85.

Conclusions: This result shows that change in voice features between conditions with and without a cognitive task can be affected by MCI.
Aims: Previous studies have reported that many activities are effective in preventing dementia. The aim of this study was to investigate the association between fractures that degrade mobility and dementia by comparing the risk of dementia between with or without fractures in senior.

Methods: This study examined a population-based matched cohort from the National Health Insurance Service–Senior Cohort data set that covers approximately half a million recipients of medical insurance in South Korea. Subjects with fracture during 2006–2012 were identified as the exposed group, and up to three individuals matched for sex, age, and index years were identified as the controls for each fracture subject. The risk of dementia for fracture was evaluated using Cox regression.

Results: During the 9-year follow-up period, 5,697 subjects with fracture (9.8%) and 14,031 subjects without fracture (8.0%) experienced dementia. Fracture was independently associated with a higher risk of dementia [hazard ratio (HR)=1.227, 95% CI=1.189–1.265] and the adjusted HR for dementia in the subjects with fracture was highest a period of 4-6 years after the initial diagnosis (HR=1.425, 95% CI=1.349–1.505).

Conclusions: This study found that fractures could be an independent risk factor for dementia in the long term. This requires consideration of mobility loss in terms of physical activity after diagnosis.
POSTERS

ON THE ASSOCIATIONS BETWEEN FOOD CONSUMPTION AND COGNITIVE DISORDERS IN A GROUP OF CUBAN OLDER ADULTS.

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National Institute of Hygiene, Epidemiology and Microbiology, Department Of Biochemistry And Phisiology, La Habana, Cuba

**Aims:** To evaluate the associations between the inadequate consumption of the different food groups and the presence of cognitive disorders such as Alzheimer's disease or Mild Cognitive Impairment in a group of Cuban older adults.

**Methods:** A cross-sectional analytical study was carried out in 402 adults older than 65 years; 40 with Alzheimer's disease (AD), 124 with Mild Cognitive Impairment (MCI) and 238 individuals without cognitive impairment in Havana. Dementia was diagnosed using the 10/66 criterion and the DSM-IV and the Petersen criterion for MCI. The diet was evaluated through a survey of weekly consumption frequency of the main food groups.

**Results:** The sufficient consumption of the different food groups among the older adults of the three groups studied was similar, except for two food groups. Individuals with AD had a significantly higher weekly meat consumption, while the consumption of cereals and meats was significantly lower in this group. High frequency of individuals from the three groups, had insufficient consumption of the different food groups: Fish: 86.1%; Fruits: 67.4%; Grains: 65.7%; Oils and fats: 61.4%; Meat, poultry and eggs: 59.5%; Vegetables: 49.5%; Sugar and sweets: 43.8%; Cereals and meats: 30.8%; and Milk and dairy products: 24.4%; respectively.

**Conclusions:** Insufficient consumption of cereals and food was inversely associated with the presence of AD, while sufficient consumption of meat, poultry and eggs was directly associated with this condition. It is necessary to continue studying this issue using semi-quantitative dietary surveys to obtain information about the diet in individuals with cognitive disorders.
Aims: The objective of this pilot study in the UHC of Nice, is to evaluate the effects of personalised virtual reality on the reduction of mood disorders in nursing homes residents with neurocognitive disorders. The secondary objectives are to assess the acceptability of the device, psychotropic drug prescription, the emotional valence and the quality of life of the residents.

Methods: A single case study is presented to illustrate the study protocol. The participant was randomised to the personalised video group. He received ten virtual reality sessions over a period of six weeks. Variables were measured before and at the end of the protocol. We performed a visual and statistical analysis of the scores.

Results: The results indicated a decrease in scores on mood scales (HDRS, GDS, IA and NPI). The interventions generated pleasure and alertness in connection with the emergence of positive memories. The headset was well tolerated by the participant. We did not observe any effect on quality of life and on the use of psychotropic drugs.

Conclusions: There is an interest in the use of reminiscence therapy using virtual reality to improve participants’ mood. Future results will allow us to conclude on the method and on its indication in nursing homes after the inclusion of 30 participants.
A COMPLEMENTARY APPROACH OF PSYCHIATRIC NURSING - SNOEZELEN THERAPY: BENEFITS FOR ELDERLY WITH DEMENTIA

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**Aims:** Dementia is a common syndrome in this stage of life and it is the cause of severe loss of functions as memory, thought process, orientation, comprehension, language, the ability to learn and complete calculation and in decision making. These losses are almost always accompanied by a deterioration of emotional control, social behavior and motivation. (WHO, 2012). According to the OCDE’s report “Health at a Glance 2017”, Portugal is the 4th country with more cases per thousand inhabitants, a fact that makes pressing and pertinent the development of intervention programs that can improve the quality of life of these individuals. The objective is to evaluate the effects and benefits of the Snoezelen therapy, on the impaired cognitive abilities, behavior, humor and social interaction of elderly individuals with dementia.

**Methods:** The sample corresponds to the institutionalized people in a Residential Unit for Elderly diagnosed with dementia. Research method: quantitative and non-experimental. Data collection is centered on the protocol of the International Snoezelen Association and other assessment tools like Mini Mental Scale Examination and the Neuropsychiatric Inventory, with the objective of introducing more rigorous data analysis.

**Results:** It was possible to reference that relatively to the evolution of cognitive capacity of the participants, the collected results weren't conclusive. However, the perception of satisfaction and well being of the patients when they performed Snoezelen therapy is evident. It is able to contribute to the promotion of quality of life.

**Conclusions:** The study suggest a trend towards positive effects in people with dementia in terms of neuropsychiatric symptoms, vital parameters and adjustment of behavior.
INFLUENCE OF WESTERN DIET AND ALZHEIMER’S DISEASE GENES IN NEURODEGENERATION, INFLAMMATION AND MEMORY FUNCTION.

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¹IBIMA Institute, Hospital Regional Universitario De Malaga, Malaga, Spain, ²Universidad de Malaga, Facultad De Ciencias, Malaga, Spain, ³IBIMA Institute, Hospital Universitario Virgen De La Victoria, Malaga, Spain, ⁴IBIMA Institute, Universidad de Malaga, Hospital Regional Universitario De Malaga, Malaga, Spain

Aims: Saturated fats and simple carbohydrates, two important components of a modern western diet, have been linked to obesity and Alzheimer’s disease (AD). Western diet has been proposed as a contributing factor in the pathogenesis of cerebrovascular and AD. Moreover, the endocannabinoid system has aroused interest, since it is involved in multiple AD-related pathologies, such as neuroinflammation and cognitive decline. Here, we explore whether chronic inflammation produced by a western diet promotes AD-like pathology in a mouse with a genetic propensity for amyloid formation and if this synergy is dependent on gut changes and endocannabinoid system alterations.

Methods: Experiments were performed on wild-type and transgenic 4-month-old 5xFAD mice with western diet or regular chow for 10 weeks. Protein expression of endocannabinoid system components, neuroinflammation markers, and beta-amyloid plaques were analyzed in the hippocampus and the gut.

Results: Based on NOR and Morris test, anxiety-like behavior and memory were altered both in the wild-type and transgenic mice when western diet was consumed. Our data suggest that there is an association between the cannabinoid receptors, cognitive function, and inflammatory response. Moreover, this association is aggravated by genetic factors which promote metabolic dysregulation. Surprisingly, the severe deterioration induced by western diet in wild-type mice suggests that an environmental factor such as diet exerts a negative impact on brain functioning.

Conclusions: Therefore, we suggest that the accumulation of beta-amyloid may not be the only cause of the worsening of cognitive impairment, but additional factors including diet, probably through a complex disruption of gut microbiota, altered metabolism and inflammation.
Aims: This study investigated the correlation of K-MMSE and K-MoCA in patients with various neurologic diseases and converted the K-MMSE and K-MoCA scores.

Methods: We recruited 979 patients with various neurologic diseases who performed K-MMSE and K-MoCA in the neurology clinic of Chung-Ang University Hospital. The neurologic diagnoses of the enrolled patients were Parkinson's disease, vascular diseases such as ischemic stroke, other neurodegenerative diseases affecting cognition such as mild cognitive impairment (MCI) and Alzheimer's disease (AD), and other neurologic disorders such as essential tremor. We compared the total score and sub-items (orientation, attention and calculation, recall, visuoconstructive function, language, and others) between K-MMSE and K-MoCA. In addition, we assessed the correlation between them at each neurologic disease. We also compared the score of K-MMSE and the conversion score of K-MoCA.

Results: There was a significant correlation between the total score of K-MMSE and that of K-MoCA (correlation coefficient rho, 0.912; p<0.01). On the comparisons of sub-items, the orientation (rho 0.958; p<0.01) and attention (rho 0.849; p<0.01) showed a strong positive correlation. Besides, the language (rho 0.710; p<0.01) and visuoconstruction (rho 0.526; p<0.01) showed moderate positive correlation. We then validated a conversion table between K-MoCA and K-MMSE in patients with various neurologic diseases.

Conclusions: In this study, we evaluated that K-MoCA was well correlated with K-MMSE. In addition, we also validated a conversion table between K-MMSE and K-MoCA in patients with various neurologic diseases. Therefore, K-MoCA can be used as an alternative cognitive screening tool substituting for K-MMSE.
A NEW NON PHARMACOLOGICAL APPROACH TO REDUCE BEHAVIORAL AND
PSYCHOLOGICAL SYMPTOMS AT HOME

A.-J. Vaillant-Ciszewicz¹, O. Said², L. Lantermino², A. Cuni², O. Guerin³
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Aims: Alzheimer’s disease and other related disorders affect 9 780 678 people. The prevalence rates of psychological and behavioral symptoms of dementia (BPSD) can reach 90%. It weakens and reduces their autonomy, quality of life as well as their self-esteem. 67% of them live at home and are looked after by their family carers. The latter often find themselves powerless when faced with their loved one’s behavioural disorders and and loss of autonomy. In order to avoid caregiver burnout, the pilot study “PsyDoMa” at the University Hospital of Nice offers personalised multidisciplinary support for those receiving care (non-drug approaches) and for their carers (psychoeducation) at home.

Methods: PsyDoMa is a study of 14 caregiver/patient dyads, set up by a team of gerontopsychologists, a nurse and an occupational therapist. During 6 months the patient will receive 3 personalised non-drug approaches sessions per week, and the carer a psychoeducation session once a week. Mederic Alzheimer Fondation support us (external evaluation). Measures included BPSD (NPI), quality of life (DQOL) psychotropic drug prescription for the patient as well as the burden experienced by the carer (Zarit scale).

Results: The expected results are a reduction in BPSD, the use of psychotropic drugs as well as an improvement in the patients’ quality of life and self-esteem.
Expected results:

For the patient:
- Improvement or maintain of their level of frailty.
- Improvement of the quality of life.
- Improvement in self-esteem and self-confidence.
- Qualitative improvement of the caregiver-patient relationship.

- Reduction of the SPCD in intensity and / or frequency.
- Reduction in psychotropic drugs intake.

For the caregiver:
- Acquisition of new skills and learning practical tools (knowledge, communication and empowerment).
- Improved mood (anxiety, doubt, guilt), self-esteem as well as self-confidence.
- Reducing the burden
**Conclusions:** Positive outcomes for carers including reductions in emotional exhaustion, improvements in mood and acquisition of new skills (pathology, practical tools) are also expected. This study attempts to respond to the ageing of the population, an increasingly important societal issue, with personalised home care.

EMOTION RECOGNITION IN SUBJECTIVE COGNITIVE DECLINE AND AMNESTIC MILD COGNITIVE IMPAIRMENT

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Aims: Ever since the domain of social cognition was included as a standard neuropsychological measure in the DSM-V, emotion recognition has attracted more attention. However, only limited data exist in patients with subjective cognitive decline (SCD) and amnestic mild cognitive impairment (aMCI), both considered as probable preclinical or prodromal stages of Alzheimer’s Disease.

Methods: Included were 41 SCD and 57 aMCI (27 single domain, 30 multiple domain) patients. Facial emotion recognition was assessed applying the Karolinska Directed Emotional Faces. Statistical analyses examined group differences via analysis of variance (ANOVA) and analysis of covariance (ANCOVA), controlling for age and gender.

Results: Patients with aMCI showed significantly more impairments in emotion recognition than SCD patients. Patients with aMCI identified less emotions correctly, especially concerning grief, joy, anxiety and anger. This effect remained stable after correction for age and gender. Additionally, there was a main effect of gender and of age with females showing significantly better emotion recognition and older patients demonstrating more impaired emotion recognition. No differences were found between single and multiple domain aMCI patients.

Conclusions: Deficits in facial emotion recognition exist concerning grief, anger, joy and anxiety in aMCI patients supporting the decision to include social cognition as a test domain in standard neuropsychological batteries. Future studies are needed to examine the underlying pathophysiological mechanisms in patients at risk for developing dementia.
COGNITIVE DYSFUNCTIONS OF OLDER PRISONERS IN GERMANY – A PILOT STUDY A

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Aims: In the context of demographic change, the number of older people in the penal system has also increased. International research points to various risk factors for the development of dementia-related syndromes including lifestyle- and health-related aspects in the prison population. Data on the prevalence of mild cognitive impairment and dementia vary greatly depending on the prison setting and sample studied and the methodology used. And to date, no data is available for the German prison population with regard to the cognitive performance of this potentially vulnerable group.

Methods: With the support of the Ministry of Justice North Rhine Westfalia, a pilot project examining the cognitive performance of older offenders was conducted. Neuropsychological examinations with established cognitive screening tools (e.g., Mini-Mental Status Test, DemTect) were conducted in 9 German prisons. Sociodemographic data as well as current or past dementia risk factors were assessed as well.

Results: 116 offenders (106 men and 10 women) aged 53 to 90 years participated in the study. With regard to global cognitive performance, about 45% showed below-average performance. Evidence of frontal dysfunction was found in 51.7% of the subjects. Executive deficits were described in about 40%.

Conclusions: These rates of cognitive dysfunctions are significantly higher than comparative data for the general population. This study highlights that further research on the steadily growing group of older prisoners regarding dementia prevention and therapy is urgently needed.
MEDICAL CANNABIS FOR ALZHEIMER’S AND PARKINSON’S DISEASE: WHAT DO ISRAELI GERONTOLOGY STUDENTS KNOW AND THINK ABOUT IT?

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Aims: The aims of the current study were as follows: 1) to assess gerontology graduate students’ beliefs about medical cannabis (MC) effectiveness for two common age-related conditions - Alzheimer’s (AD) and Parkinson’s disease (PD); 2) to assess students’ beliefs and attitudes toward MC; 3) to explore associations linking background characteristics, MC-related attitudes and beliefs, and beliefs about the MC effectiveness for AD and PD.

Methods: A sample of 104 (84 women and 20 men) gerontology graduate students voluntarily participated in the anonymous online survey.

Results: The vast majority (95%) of the participants indicated they had no formal education about MC and reported being unprepared to answer clients’ MC-related questions (84.6%). Most participants believed that MC is effective for use with AD (70.2%) and PD (80.8%) patients. Participants reported favorable beliefs about MC benefits, concerns about risks, the need for training, and positive attitudes toward recreational marijuana use legalization. Prior cannabis use (e.g., self-use, friends, or family) was associated with more positive beliefs about MC benefits, risks, and its legalization for recreational purposes. Prior cannabis use was the only factor associated with the belief that MC is an effective therapy for AD or PD patients.

Conclusions: The study findings show the need for students’ MC education in order to provide future gerontology service providers with the necessary knowledge and ability to address clients’ questions about MC use. Efforts to develop curricula and training programs need to be promoted.
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Aims: Delirium is reported to be more common in patients with Dementia with Lewy bodies (DLB) both prior to and after diagnosis. The aim was to analyze delirium frequencies in a dementia cohort in Norway, comparing subgroups of subjects with DLB and Alzheimer’s disease (AD).

Methods: The Dementia Study of Western Norway included 247 persons with mild dementia (MMSE ≥ 20), (Clinical Dementia Rating Scale (CDR) =1), who were followed annually until death. Delirium was retrospectively diagnosed through chart review assessing all available information in 341 acute or planned hospital admissions to both psychiatric and somatic wards from 5 years before dementia diagnosis until death. 23 DLB and 55 AD or mixed AD and vascular dementia patients were included in the present analysis. Pearson Chi-Square test was used to compare the groups.

Results: Delirium was recorded in 101 (29.6 %) hospital admissions. There was no significant difference between delirium frequencies in the DLB and AD groups. Moreover, there was no significant difference between delirium frequencies before or after dementia diagnosis in the DLB and AD groups.

Conclusions: It is challenging to diagnose delirium through chart review due to recurring poor documentation in the hospital chart. Many delirium episodes occurred in moderate and advanced stages of dementia. Thus, the diagnoses will, in many cases, be uncertain and delirium frequency is probably underdiagnosed in this analysis. More studies are necessary to ascertain whether delirium is more common in DLB patients, or if DLB symptoms are misinterpreted as delirium.
CLINICAL TRANSITION TO ALZHEIMER’S DISEASE AND PREDICTORS IN INDIVIDUALS WITH MILD COGNITIVE IMPAIRMENT

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Aims: Clinical transition from mild cognitive impairment (MCI) to Alzheimer’s disease (AD) is still not well understood. This study was to evaluate risk and predictors for AD onset in individuals with MCI.

Methods: Individuals with MCI were identified from the US Veterans Affairs healthcare database between January 2006 and November 2021. MCI or AD was defined by ≥2 ICD codes recorded >30 days apart. Probabilities of clinical transition to AD over 15 years were described. Rate of transition to AD or death was estimated using multi-state Markov modeling (MSM) and predictors associated with AD transition were estimated with Cox regression only in those who developed AD.

Results: 93,815 individuals aged 50-90 years with MCI were identified, and 4,180 of them developed AD. Probabilities of transitioning to AD within a year ranged from 2.5% in 2013 to 10.3% in 2021 with a mean of 5.1%. MSM estimated MCI-to-AD transition rate was 2.7% (95%CI:2.6%-2.8%), and AD-to-death 19.5% (18.4%-20.8%). Risk of AD transition significantly increased with congestive heart failure (hazard ratio=1.28,1.02-1.60), depression (1.18,1.02-1.36), diabetes (1.18,1.05-1.33), hypothyroidism (1.30,1.05-1.61), and hypercholesterolemia (1.16,1.03-1.30). This risk decreased among nursing home residents (0.87,0.79-0.95).

Conclusions: Mean probability of clinical transition from MCI to AD is 5.1% per year among Veterans, and the risk of transition increases with depression or cardiovascular disorders and decreases with nursing home residency. One death occurs in every five Veterans with AD yearly over the study period.
Aims: To investigate the current status of long-term care services for patients with dementia and lifetime medical costs for dementia in South Korea.

Methods: This study utilized the National Health Insurance Service-National Health Information Database (NHIS-NHID) from January 2013 to December 2017. The prevalence and incidence of dementia was estimated by extracting people who were diagnosed and treated with dementia (age ≥ 45 years) from the database. The use of long-term care services for the elderly with newly diagnosed dementia was also investigated. Additionally, the lifetime medical expenses for dementia were estimated using data on single year’s medical costs, population data, and a life table from Statistics Korea.

Results: The prevalence of dementia increased over three years from 2015 to 2017, while the incidence of dementia gradually decreased. Among the patients with newly diagnosed dementia, approximately 30% used the long-term care services, while 4th graders accounted for the highest proportion every year. The older the age and the lower the income quartile, the shorter the time it took to apply for long-term care services after diagnosis of dementia. The total medical expenses per capita increased steadily every year, and the lifetime medical expenses were higher for females than males. Half of the lifetime medical costs of dementia occurred after 67 years of age for males and 83 years for females.

Conclusions: This study suggests that medical, social, and political measures are needed to effectively manage long-term care service recipients and prepare for rising medical costs for dementia.
Aims: To establish a nationwide programme in Luxembourg that specialises in dementia prevention using personalised lifestyle interventions which aim to prevent or slow down the cognitive decline in at-risk individuals.

Methods: The target population is comprised of individuals with a subjective or mild cognitive impairment. The participants were pre-screened and referred to by a treating physician before undertaking an elaborate cognitive evaluation and a structured dementia risk factor assessment by a neuropsychologist. Based on these findings, participants were offered cost-free interventions specifically adapted to their risk profile. Both cognitive status and risk profiles were reevaluated based on annual follow-up visits to assess potential changes and to ensure the adherence to the offered precision prevention measures.

Results: Establishing a nationwide prevention network, which successfully reached its target population, allowed for the collection of extensive clinical and neuropsychological data during first (N=248) and second visits (N=73) respectively. This includes information regarding the evolution of participants' cognitive status and related risk factors as well as the general adherence to the programme and the acceptance of different lifestyle interventions. Preliminary findings point towards an adherence rate of 87%, with differing acceptance levels for physical, cognitive, nutritional, and psychological interventions.

Conclusions: We provide evidence that implementing a national dementia prevention programme providing personalised health care interventions, is feasible and can be sustained longitudinally with a high level of adherence. The insights gathered by our programme may inform about how to reduce dementia risk factors and allow for the integration of novel preventive measures into the regular healthcare system.
AN INCLUSIVE RESEARCH MODEL: ADDRESSING RECRUITMENT OF UNDERREPRESENTED POPULATIONS IN ALZHEIMER’S DISEASE (AD) CLINICAL TRIALS

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Aims: To present the Global Alzheimer’s Platform Foundation’s (GAP) inclusive research model that addresses recruitment challenges of underrepresented populations in Alzheimer’s disease (AD) clinical trials.

Methods: GAP has developed an inclusive recruitment model that provides underrepresented populations with awareness, knowledge and access to AD clinical trials. The model begins with community mapping, which then informs targeted engagement efforts with established and trusted organizations that serve underrepresented communities. This ongoing model was initially deployed and tested in April 2020 for GAP’s Bio-Hermes study, which seeks to examine and validate both blood and digital biomarkers in healthy, MCI, and probable AD participants. GAP has committed to ensuring that 20% of the participants are from underrepresented populations, specifically Black/African American and Hispanic/Latino individuals across 12 GAP-Net sites.

Results: As of September 10, 2021, Bio-Hermes has enrolled a total of 323 participants, 57 (18%) from underrepresented populations. Final results will be shared during the 2022 AD/PD Conference. Community mapping has successfully resulted in identification and relationship development between GAP-Net sites and community-based organizations, minority-owned media, local leaders, and HCPs that serve traditionally underrepresented communities.

Conclusions: GAP’s inclusive research program is designed to increase recruitment and enrollment of underrepresented people in AD clinical trials. Bio-Hermes is on track to meet its target enrollment of 20% from these populations. To further improve access, awareness and trust among underrepresented populations, GAP is planning to include the following additions to its inclusive research model: • Mobile units to broaden outreach efforts • New AD site in a community with 98% Hispanic/Latino ethnicity
LEVEL OF PERCEIVED SOCIAL SUPPORT IS POSITIVELY ASSOCIATED WITH AGE AND EDUCATION IN PEOPLE WITH DEMENTIA

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Aims: The study related to social health in people with dementia is lacking, particularly in Indonesia. This study aims to investigate factors associated with the level of perceived social support in people with dementia.

Methods: This was a cross sectional study. We recruited 54 patients with dementia who came to the Memory Clinic of Dr Sardjito General Hospital, Yogyakarta, Indonesia. We recorded the sociodemographic characteristics, as well as measured their cognitive functions using Montreal Cognitive Assessment-Indonesia version (MoCA-Ina) and the level of perceived social support using Personnel Resources Questionnaire-2000 (PRQ-2000). Multivariate linear regression was performed to investigate factors associated with the level of perceived social support in patients with dementia.

Results: The mean age of participants was 68 years old and most of them were male (64.81%). The mean score of MoCA-Ina was 12.54±7.52 indicated the performance of cognitive function of the participants was poor. However, with the mean score of PRQ-2000 was 81.54±14.65 which indicated the perceived social support of the participants was quite good. Multivariate analysis showed that PRQ-2000 was positively associated with age and education (B=0.8029, 95%CI=0.4075-1.135 and B=1.464, 95%CI=0.1223-2.807, respectively). However, no correlation found between PRQ-2000 and the level of cognitive function.

Conclusions: The level of perceived social support in people with dementia was positively associated with age and education. This could be attributable to the fact that advanced age and higher education may lead to positive perception towards life. Future studies is needed to explore more about the social health in dementia, particularly how it influence the quality of life.
Aims: There are nearly 5 million dementia in Japan, and the number is expected to increase in the future. In this situation, it is natural that new legal issues will be occurred. In the event of an accident involving the elderly, the elderly person himself is first held legally liable. However, when the elderly have dementia, the elderly’s family may be held accountable for supervision. In Japan, an elderly man with dementia died after entering a railroad track in 2007, and his elderly wife and son were held accountable for the damage by JR Tokai that delayed the train. However, the legitimacy of hold elderly families with dementia accountable for supervisors was disputed. This study introduces the situation in Japan and raises the discussion about the responsibility of the family.

Methods: I used the internet and databases to search for and analysed government guidelines and related literature in Japan.

Results: March in 2016, Supreme court denied family’s liability to pay damages. Because it is difficult to expect his wife take care of him as appropriate supervisor actually in this case.

Conclusions: Modern tort law asks whether the perpetrator was intentionally or negligently, and pays damages to the victim. The Japanese media raised questions about whether the elderly and their families could be blame, and the Supreme Court tried to respond to the accusations. As the number of accidents caused by dementia increases worldwide, it is expected that a system will be established to solve the problem by using a non-trial method, such as the insurance system.
Aims: Magnetoencephalography (MEG) is sensitive to changes in brain activities associating with cognitive decline. We provide checkup service to assess the risk of dementia using MEG. The risk is estimated using two algorithms: Classical approach (CA) and Artificial intelligence approach (AIA). Estimated risk is expressed in four levels scores from A “likely to be healthy” to D (“cognitive decline suspected”). In this study, we verified the accuracy of the estimations by comparing with clinical diagnosis.

Methods: Thirty-one participants, who underwent the checkup from August 1, 2020 to February 17, 2021, and the estimated risks were compared with the diagnosis. This study was conducted under the approval of the local Ethics Committee.

Results: Sixteen participants were considered as healthy aging (the “healthy group”), and the others were noted to have some cognitive problems (the “patient group”). In the healthy group, CA assigned a participant to A and 15 participants to B, while AIA assigned eight to A, seven to B and one participant to D. In the patients group, CA assigned 10 to B five to C, while AIA assigned five to A, two to each of B and C, and six to D. Assuming that C-D is considered positive, the sensitivity and specificity were 33% and 100% for CA, 53% and 94% for AIA.

Conclusions: These results suggest that the checkup service was good for high specificity rather than sensitivity.
Aims: Identifying mild cognitive impairment (MCI) is important during aging because it has been related to an increased risk of developing Alzheimer’s Disease and other dementias. The early detection of cognitive impairment through neuropsychological assessment is essential. Participation in cognitively active activities during aging is associated with changes in the pattern of cognitive decline in older adults due to an increased cognitive reserve. Thus, active older adults might present a diagnostic challenge in conditions such as MCI and AD, as pathological changes might go undetected in the neuropsychological assessment.

Methods: Regression-based normative data were calculated for 8 neuropsychological tests 118 highly cognitively active adults aged 55 or older (SABIEX Normative Data). Age, sex, and educational level were included as predictors. Interdependent variables were also included in tests with related tasks, such as TMT (the predictive model of TMT-B including the TMT-A scores). The number of low scores was compared using population-based against SABIEX normative data.

Results: The number of low scores using population-based and SABIEX was 1.94 (SD= 1.54) and 0.95 (SD= 1.33) respectively. The proportion of people with at least one low score (NEURONORMA=53.5%; SABIEX=69.3%) was significantly different between normative data (McNemar χ²=8.5, p<.004). There was poor agreement between B (Kappa=0.37; p<.001) in the classification of participants with low scores. MMSE scores differed only with SABIEX normative data.

Conclusions: The norms obtained in the general population could be less sensitive to identify low scores in cognitively active older adults, increasing the rate of diagnostic errors.
Aims: As a method for detecting dementia, the Mini Mental State Examination (MMSE) is commonly used in clinical practice, but it requires a doctor to perform the test, and it is not easy. On the other hand, it has been reported that dual tasking, in which two things are done simultaneously, is effective in detecting dementia. In this study, we verified the effectiveness of dual tasking of performing calculations while walking.

Methods: Elderly people aged 60 or older were recruited and their cognitive ability was tested by MMSE. Then, the walking speed was measured during normal walking and walking with calculations. The calculations consisted of sequentially subtracting 7 from 100, and the answers were given aloud. Overall, 73 males and 147 females participated in this study.

Results: There was a significant difference in walking speed between normal walking and walking with calculations ($t(219)=19.66$, $p<.01$). No correlation was found between normal walking speed and MMSE score for either gender. There was a significant weak positive correlation between walking speed with calculations and MMSE score for males ($r=0.34$, $p=.0034$). There were no gender differences in MMSE score, normal walking speed, and calculation task score in
Conclusions: For males, walking speed with calculations tended to decrease with cognitive tendency. In general, males are said to be better at single-tasking and females at multi-tasking, suggesting that the difference is more pronounced in the cognitive tendency. Therefore, it should be noted that the sensitivity to females may be lower in the detection of dementia by dual tasking.
EFFECTS OF TDCS ON BASIC COGNITIVE ABILITIES OF PATIENTS WITH INITIAL DEMENTIA

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Aims: Transcranial direct current stimulation (tDCS) is based on the ability to modulate cortical activity and drive neuroplasticity mechanisms through low intensity continuous electrical currents in the brain. Studies point to benefits in the performance of cognitive functions for dementia patients. This study aims to verify whether the application of the tDCS technique in patients with initial dementia facilitates improvement in the scores of basic cognitive skills assessment tests.

Methods: Nine patients with a diagnosis of dementia were evaluated using MMSE, T@M and direct digits. 10 sessions of 20 minutes of tDCS were applied. In the stimulation protocol, the constant current was 2mA, the anode was positioned on the left frontotemporal region (F7) and the cathode on Fp2, right supraorbital area. The patients were evaluated before and after applying the intervention. Paired-Samples T Test were applied.

Results: Significant differences were obtained when comparing the means before and after the intervention in the tests: MMSE (p = .042; Xpre = 23.22, Xpost = 25.88), T@M (p = .018; Xpre = 27.11, Xpost = 32.33) and direct digits (p = .010; Xpre = 6.22, Xpost = 8.22).

Conclusions: The efficacy of tDCS for the improvement of basic cognitive abilities in patients with mild dementia is concluded; This could be a technique that facilitates the maintenance of other cognitive functions. In addition, the comparison with a control group to which sham is applied is proposed as a future line.
Aims: Autobiographical memory consists of a person’s personal history and contributes to building a feeling of identity and continuity. Detecting changes in Alzheimer's disease in the early stages is one of the main challenges of current research. Autobiographical memory involves the retention and retrieval of experiences from one’s personal past. For this reason, it is important to know the changes that occur in autobiographical memory during Alzheimer's disease. This study evaluated semantic and episodic autobiographical memory in Alzheimer's disease patients and compared their scores within one year to analyze possible differences.

Methods: Autobiographical Memory Interview was applied to 14 patients with Alzheimer's disease. Autobiographical Memory Interview is a semi-structured interview used to assess memory retrieval in two domains: personal semantic and autobiographical incidents that are considered episodic. Paired-Samples T Test were applied.

Results: Autobiographical Memory scores of the participants who were assessed one year apart were compared. The analysis of means showed differences for semantic autobiographical memory (p = .012; Xpre = 39, Xpost = 31.5) with a significant decrease; episodic autobiographical memory also showed a significant decrease in the means obtained p = .008; Xpre = 11.7, Xpost = 7.6).

Conclusions: In patients with Alzheimer’s disease, a significant deterioration is observed in both semantic and episodic autobiographical memory.
EFFECT OF NANOMICELLE CURCUMIN ON QUALITY OF LIFE AND SLEEP IN PATIENTS WITH PARKINSON’S DISEASE: A DOUBLE-BLIND, RANDOMIZED, AND PLACEBO-CONTROLLED TRIAL

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Aims: Considering the evidence indicating the neuronal protective effects of curcumin in previous studies, this double-blind, randomized, placebo-controlled, and parallel-group trial was aimed at exploring the possible nanomicelle curcumin (SinaCurcumin®, nano-micellar soft gel)-mediated impact on sleep, fatigue, and quality of life (QoL) in patients with Parkinson’s disease (PD).

Methods: A sample of 50 PD patients were recruited and randomly divided into experimental (25) and control groups (25). Sleep quality, fatigue, and QoL were assessed based on the Pittsburgh Sleep Quality Index (PSQI), Fatigue Severity Scale (FSS), and the Parkinson’s Disease Questionnaire–39 (PDQ-39), respectively, at the beginning and the end of the study. The groups were treated for three months by 80 mg of nano-micellar soft gel twice a day.

Results: Nanomicelle curcumin significantly increased sleep quality and QoL compared with placebo (P values = 0.0001 and 0.0002, respectively) in PD patients. This significant difference has not influenced by the duration of the disease, the severity of disease progression (Hoehn & Yahr scale), and the cumulative dose of levodopa. This supplement did not have a significant effect on the fatigue severity of patients compared to placebo.

Conclusions: It has proposed that the nanomicelle curcumin can be used to improve sleep quality and QoL in PD patients.
EXERCISE HABITS OF PATIENTS WITH PARKINSON’S DISEASE DURING THE COVID-19 PANDEMIC

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Aims: Exercise is critical in maintaining physical and mental health for people with Parkinson’s disease (PD) (1). Our objective was to examine exercise habits in people with PD during the COVID-19 pandemic. Further, we explored the self-reported health-related quality of life, mental health, and coping skills of the population surveyed.

Methods: This cross-sectional study involved completion of an electronic survey containing 75 questions exploring exercise habits, the Parkinson Disease Questionnaire-39 (PDQ-39), the Patient Health Questionnaire (PHQ-9), and the Coping Self Efficacy Scale-13 (CSES-13). Patients with PD, aged 18-95, who were members of the local Parkinson’s foundation and were independent with activities of daily living were invited to participate.

Results: 142 of 160 study participants (aged 72.5 years + 7.9; 45.3% male) met inclusion criteria. 52.1% of respondents reported exercising regularly (> 3 times/week) pre-pandemic, while 59.3% reported exercising regularly during the pandemic. 23.9% of respondents used technology for exercise pre-pandemic, while 82.9% use technology for exercise during the pandemic, and 48.2% relied on online exercise as their main mode of exercise. Although most respondents preferred in-person exercise, 80.7% reported some stress-relief with online classes and 65.8% reported a positive association with mood. 83% indicated that they would continue online exercise classes once in-person classes became more readily available.

Conclusions: Online exercise resources are important tools for patients with PD during the pandemic and will likely continue to be utilized post-pandemic. Continued optimization and access to online exercise classes for people with PD is warranted. 1) Hackney ME, Earhart GM. [...] Doi: 10.1016/j.parkreldis.2009.03.003.
AMYOTROPIC LATERAL SCLEROSIS WITH BACKGROUND OSTEOARTHRITIS: A CASE REPORT

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Aims: To highlight the problems of Amyotrophic lateral sclerosis patients and the need for more research in order to optimise caregiver support. Thus, relieving patients’ suffering while extending survival.

Methods: This paper is a case report. Meticulous history taking, thorough clinical examination examination and detailed documentation of the clinical findings were performed. Continuous follow up and assessment of treatment progress with documentation of outcomes was also done. A careful search and review of existing medical literature and comparison with the findings in this case was performed.

Results: The patient is 62-year-old woman who presented with a 4-year progressive weakness and atrophy of the muscles of the 4 limbs (worse in upper limb). She also had mild dyspnea (9 months) and slurred speech (3 months). Functional use of all limbs and her cardiorespiratory function have been significantly negatively impacted by her condition. She is unable to perform Activities of Daily Living. There is difficulty in maintaining an erect posture while standing and sitting positions (she slouches). Slurring of speech was also observed. She was diagnosed of right knee osteoarthritis and cervical spondylosis, 6 and 4 years ago respectively. Her management included oral and topical analgesics alongside physiotherapy interventions. She is receiving multidisciplinary care currently.

Conclusions: With its poor prognosis, this disease has profound implications on the quality of life of patients. Depression is common in this group. More research into discovering the cause of the disease, preventive strategies and the definitive treatment. Better techniques for upgraded caregiver support should be advocated and researched.
QUALITY-OF-LIFE IMPROVEMENT IS ASSOCIATED WITH SOME PERSONALITY DIMENSIONS IN FLUCTUATING PARKINSON’S DISEASE PATIENTS AFTER CONTINUOUS SUBCUTANEOUS APOMORPHINE INFUSION

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Aims: We aim to evaluate associations between Quality of Life (QoL) amelioration after six months of Continuous Subcutaneous Apomorphine Infusion (CSAI) and personality dimensions in Parkinson’s Disease (PD) patients.

Methods: In PSYCHO-PERF study, twenty-nine patients awaiting CSAI were included and twenty-six finished the protocol after six months of CSAI. Associations between the seven personality dimensions (evaluated by the “Temperament and Character Inventory” (TCI) before CSAI) and percentage of QoL evolution (assessed by the PDQ-39 (Parkinson’s Disease Questionnaire-39)) after six months of CSAI were evaluated with linear regression models.

Results: Better PDQ-39 scores evolution after six months of CSAI were associated with higher Reward Dependence scores (FDR-corrected p.value < 0.05); while better PDQ-39 scores before CSAI tended to be associated with lower Harm Avoidance scores (FDR-corrected p.value = 0.05).

Conclusions: PD patients with higher Reward Dependence scores before treatment had a better QoL outcome after six months of CSAI. A better social adjustment linked to Reward Dependence may explain this result, while anxio-depressive state before treatment may be related to higher Harm Avoidance scores and worst QoL. These results could be used in clinical practice to better prepare and accompany PD patients before and during CSAI establishment through specific therapeutic education programs focusing on Reward Dependence temperament.
A SYSTEMATIC REVIEW OF NEUROPSYCHIATRIC SYMPTOMS IN PARKINSON'S DISEASE

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Aims: The objective of this systematic literature review was to describe the prevalence and nature of neuropsychiatric symptoms (NPS) among patients with early PD. Additional objectives were to identify the most common NPS among patients, to define the cognitive domains impaired in patients, and to explore the relationship between NPS and current approaches to the treatment of PD.

Methods: We identified reports of NPS in patients with PD of at least 3 years disease duration. Three databases – PubMed, Scopus and Dialnet – were searched for relevant literature published between 2010 and 2020. Search terms included 'neuropsychiatric' and 'parkinsons'. Predefined exclusion criteria were applied prior to a descriptive analysis of the literature base.

Results: In all, 87 unique reports were identified and 26 met inclusion and exclusion criteria. These included 6259 patients with PD (male: 57.3%; mean age: 66.4 years; mean disease duration: 7.4 years). The most frequent NPS were mood symptoms, particularly apathy, depression, anxiety, as well as psychosis and impulse control disorders (ICD). Treatment with dopamine agonists was identified as an important risk factor for ICD. Co-occurrence of NPS and cognitive dysfunction was also evidenced in a number of studies. Patients with more significant cognitive deficits and higher levels of NPS appeared to be of older, with a longer disease duration and to have more severe motor symptoms.

Conclusions: NPS, most commonly mood disorders, are a frequent manifestation of early PD. Dopamine-replacement therapy may be associated with the emergence of some NPS.
ASSOCIATION BETWEEN BODY MASS INDEX AND THE ALL-CAUSE MORTALITY IN PARKINSON’S DISEASE: A NATIONWIDE POPULATION-BASED COHORT STUDY

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Aims: Weight loss could occur in the prodromal stage or course of Parkinson’s disease (PD) progression. Therefore, the objective of this study was to investigate the association between baseline BMI and BMI change at PD diagnosis and all-cause mortality rate in patients with PD.

Methods: The nationwide population-based retrospective cohort study was conducted using the Korean National Health Insurance System - Elderly Cohort data. We selected PD patients with a primary or secondary diagnosis of ICD-10 code G20. Health screening data within 2 years of PD diagnosis were selected for baseline BMI, and the most recent follow-up data from the baseline were also selected to calculate BMI change. BMI was calculated as the weight divided by height squared (kg/m²) and categorized into five groups: < 18.5, 18.5–23.0, 23–25, 25–30, and ≥ 30 kg/m².

Results: In total, 2,703 patients with PD were enrolled. There was a significant inverse relationship between BMI and mortality: as BMI at PD diagnosis increased, mortality was significantly reduced (<18.5: HR, 1.872, 95% CI, 1.338–2.494; 23–25: HR, 0.695, 95% CI, 0.546–0.886; 25–30: HR, 0.644, 95% CI, 0.476–0.869; ≥30: HR, 0.396, 95% CI, 0.165–0.950). However, BMI change by 3%, and 5 %, and mortality did not show significant relationship.

Conclusions: We found that BMI at PD diagnosis was significantly related with mortality in patients with PD, whereas BMI changes showed no relationship with mortality.
HIGH INTENSITY INTERVAL TRAINING INCREASES ELECTROENCEPHALOGRAPHIC MOTOR RELATED CORTICAL POTENTIAL AND BLOOD LEVEL OF BRAIN DERIVED NEUROTROPHIC FACTOR IN PARKINSON'S DISEASE PATIENTS

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Aims: Our study evaluated the effects of 12-week high intensity interval training (HIIT) on the electroencephalographic motor related cortical potential (EEG-MRCP) as well as on the blood level of brain-derived neurotrophic factor (BDNF) in Parkinson's disease (PD).

Methods: Mild to moderate PD patients were tested. The trained group (PD-TR, n=14) besides receiving usual care underwent 12-week of HIIT program on a cycle ergometer and the non-trained group (PD-NTR, n=13) received only usual care. The 12-week HIIT program consisted of three 1-hour training sessions weekly. Both groups were tested while their medication OFF-phase before and after the 12-week HIIT period. The following assessments were performed: (i) EEG-MRCP amplitude from the electrodes in locations of: PFC, DLPFC, SMA, motor and premotor cortex, and (ii) blood serum BDNF level using ELISA. EEG signals were recorded using a 128-channel system during performance of one hand index finger extension task.

Results: Comparisons of data between post- vs. pre-HIIT testing sessions indicated in the PD-TR group a significant increase of: (i) the amplitude of EEG-MRCP in the PFC, ipsilateral DLPFC, SMA and in ipsilateral motor cortex brain areas, as well as (ii) the blood BDNF level. There was no difference between the pre- and post-HIIT testing sessions in any parameter in the PD-NTR group.

Conclusions: Following 12-week HIIT program, the trained PD patients exhibited an increase of: (i) the EEG-MRCP amplitude mainly in prefrontal cortex and SMA locations and (ii) the blood BDNF level, what supports a beneficial neuroplastic impact of HIIT in PD treatment.
CUMULATIVE EFFECTS OF COMBINED RTMS AND PERTURBATION TREADMILL TRAINING IN GAIT PERFORMANCE IN PARKINSON DISEASE

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Aims: Abstract Previous studies have demonstrated repetitive transcranial magnetic stimulation (rTMS) combined with rehabilitation therapies might enhance the therapeutic effects. Objective: The study aimed to determine whether priming with 10Hz repetitive transcranial magnetic stimulation (rTMS) will enhance the benefits from perturbational treadmill training in people with Parkinson's disease (PD).

Methods: In this randomized double-blind, placebo-controlled trial eighteen individuals with PD were randomized to receive four sessions (10Hz, or sham) rTMS followed by perturbational treadmill training. All participants received a single session of perturbational treadmill training. The experimental group (n=9) walked with perturbations produced by small three-dimensional tilting movements after they received a single session of rTMS (10Hz). The control group (n=8) performed treadmill training with sham rTMS. All participants were assessed at baseline and the end of the intervention. Outcome measures were self-selected comfortable overground walking speed (10-Meter-Walk-Test), timed up-and-go test (TUG), and center of pressure sway velocity (vCOP) and sway area (aCOP).

Results: The experimental group significantly improved in overground walking speed after the intervention, compared to the control group (p = 0.01). By contrast, postural control measures did not differ significantly between groups after intervention. only the experimental group showed significantly reduced lateralization variability of gait (p <0.05)

Conclusions: Combined rTMS plus perturbational treadmill training led to beneficial training in gait performance. The cumulative benefit may be due to motor cortex excitability enhancement. Further research for investigating long time effect is warranted.
DANCING USING THE FELDENKRAIS METHOD IMPROVES MOTOR, NON-MOTOR SYMPTOMS AND GAIT IN PARKINSON’S DISEASE: A 12-MONTH STUDY

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Aims: Dancing intervention based on the Feldenkrais method improves balance and gait in the elderly population and patients with cognitive impairment. However, the effects of dancing remain unclear in patients with Parkinson’s disease (PD). The aim of this study was to assess the effects of dancing using the Feldenkrais method on motor and non-motor symptoms, health-related quality of life (QoL), and objective parameters of gait at the time of intervention and at the end of the 1-year study period.

Methods: This was a single-arm study in which 12 subjects with PD who received a dancing intervention based on the Feldenkrais method were recruited prospectively during a 6-month period. Objective motor scales, gait analysis, and questionnaires for non-motor symptoms were evaluated at baseline and at 3, 6, and 12 months. During follow-up, 3 subjects dropped out.

Results: Dancing improved motor scale (Unified Parkinson's Disease Rating Scale (UPDRS) and Tinetti scale) scores and improved gait disturbance (gait velocity and step length) without increasing levodopa equivalent dosage. Furthermore, dancing decreased non-motor scale (Non-Motor Symptoms Scale and Montgomery-Asberg Depression Rating Scale) scores and improved QoL.

Conclusions: Although further studies using a double-arm design are needed to support our results, the findings suggest that dancing intervention is a complementary management method for PD.
Aims: One of the non-motor features of idiopathic Parkinson disease (IPD) is sexual dysfunction (SD) which is under-recognized and, consequently, under-treated. Objective: This study aimed to evaluate SD in IPD patients

Methods: The study was conducted on 67 patients with IPD and 30 age and sex healthy subjects who served as control group. All participants were subjected to sexual functions assessment by using Arabic version of sexual health inventory for men (SHIM) and Arizona sexual experience scale (ASES), Mini Mental State Examination (MMSE) and Beck Depression Inventory (BDI). While the severity of IPD patients were assessed according to modified Hoehn and Yahr scoring scale.

Results: There were no difference between IPD patients and controls as regards presence of partner, MMSE, hypertension, diabetes mellitus, dyslipidemia or smoking. While; BDI score was significantly higher in IPD. The rate of sexual dysfunctions among our patients was 64% compared to 30% among control group. The total score and sub-scales of ASES were significantly higher in IPD patients than controls. The mean score of SHIM was lower in male PD patients than male control. SD showed significant correlation with severity of the IPD irrespective of other variables including age of the patients, sex, durations of the disease, hypertension, diabetes, dyslipidemia, smoking and dose of L-dopa.

Conclusions: Sexual dysfunction is a common under-rated feature in IPD patients and should be investigated carefully. As it is important one of the non-motor symptoms that correlates with the disease severity.
Aims: Parkinson’s disease (PD) is the second most common neurodegenerative disorder, after Alzheimer’s disease. Parkinson’s disease main four clinical features include muscle rigidity, tremors, and bradykinesia and gait. Also there is secondary features like depression, emotional change, dementia, constipation, postural hypotension, changes in speech, skin problem, and dysphagia. The study aims to identify the clinical features of Parkinson disease in Sudanese Parkinson’s patient.

Methods: A descriptive cross sectional clinic based study during the period of January 2020 to January 2021. Data was collected using unified Parkinson disease rating scale via trained doctors on interview based clinic setting with full medical history and physical examination.

Results: This study includes 35 patients with Parkinson disease 25 (71.4%) were male and 10 (28.6%) were female. The most common age of the patients ranges from 61 to 70 years (34.5%). Bradykinesia was found to be the most common symptoms (85.7%) more than rigidity (82.9%), tremor (74.3%) and postural gait instability (71.4%).

Conclusions: This study has identified the most common clinical features of Parkinson’s disease. The present study confirms previous findings, notwithstanding the relatively limited sample, this work offers valuable insights into the clinical presentation of Parkinson disease in Sudanese patient.
Aims: The basic epidemiology of institutionalization (the need for long-term care in a nursing home or other facility) in parkinsonism is unclear. We aimed to identify the incidence of, and risk factors for, institutionalization in Parkinson's disease (PD) and atypical parkinsonism (AP).

Methods: We analysed data from a prospective population-based incidence cohort of parkinsonism in North-East Scotland (the PINE study). 556 participants (PD, N=200; AP, N=98; controls, N=258), recruited between 2002 and 2009, were prospectively followed life-long to May 2020 with data collection on place of residence. We calculated the incidence of institutionalization and determined baseline predictors (measured at diagnosis) of institutionalization using Cox regression.

Results: Over a median follow-up time of 9.3 years in PD and 4.4 years in AP, 70 with PD, 53 with AP, and 43 controls were institutionalized. The incidence rates of institutionalization in PD, AP, and controls were 5.1, 20.8, and 1.8 per 100 person-years respectively. The median time to institutionalization was 11.8 years in PD, 3.5 years in AP. Multivariable Cox regression showed that AP [HR versus PD (95% CI)=3.02 (1.84,4.96)], increasing age [HR for 10-year increase (95% CI)=1.89 (1.43,2.51)], poorer cognition [HR for MMSE<24 versus MMSE>27 (95% CI)=2.60 (1.43, 4.72)] were independently associated with higher hazards of institutionalization. Sex, Charlson co-morbidity index, smoking history, living alone, UPDRS part 3 (motor) score, and Schwab and England scale, were not associated with institutionalization.

Conclusions: Institutionalization is much more frequent in parkinsonism, particularly AP, than in controls. AP, older age, and poorer cognition were independent baseline predictors.
THE CANADIAN OPEN PARKINSON NETWORK

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Aims: The Canadian Open Parkinson Network (C-OPN) is a pan-Canada initiative that bridges people, data and resources to accelerate new discoveries in Parkinson’s disease (PD) research. C-OPN operates under the principals of Open Science, and aims to facilitate rapid sharing of data and samples with Canadian and international investigators and industry partners.

Methods: Eight of Canada’s top universities and movement disorders research centres from British Columbia, Alberta, Ontario and Quebec are part of C-OPN. The Network collects de-identified clinical data with comprehensive information about each participant’s family history, lifestyle and environment, along with details of their PD symptoms and medications; test results (e.g. MoCA, MDS-UPDRS); and a biobank with biomaterials extracted from blood samples, including DNA, peripheral blood mononuclear cells (PBMCs) and serum.

Results: Since its launch in June 2020, C-OPN has met the following milestones: i) established research activities lead by C-OPN coordination teams at each site; ii) instituted a bilingual REDCap online database; iii) currently developing LORIS platform to facilitate an open, user-friendly, data-sharing platform with researchers (expected launch: Winter 2022); and iv) begun setting-up collaborations and supporting studies that will drive the field of PD research into the next dimension. To date, the project has enrolled 682 participants across Canada. Furthermore, studies from several Canadian Universities as well as the University of Oxford (UK) have worked with C-OPN to increase their recruitment through our Network.

Conclusions: In summary, C-OPN seeks to support cutting-edge, multi-disciplinary and multi-site PD-related research studies across Canada and around the world.
EFFECT OF EXERCISE ON THE COGNITIVE ABILITIES OF ALZHEIMER PATIENTS

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Aims: Alzheimer’s is one of the most common degenerative cerebrovascular diseases that is associated with cognitive impairment and is most commonly found in old age. The aim of this study was to investigate the effect of aerobic exercise program with fixed bicycle on cognitive abilities of patients with Alzheimer’s dementia.

Methods: Materials & Methods The present research was a quasi-experimental study with pre-test posttest design with control group that was performed on 14 patients with Alzheimer’s; they had a mean age of 67 years old membered in the Alzheimer’s Association of Iran in 2015, who were selected by purposive sampling method and randomly divided into experimental (n=7) and control (n=7) groups. All patients were evaluated in two stages of pre-test and post-test with MMSE and MoCA tests. Then, for the experimental group, the exercise program was performed for 3 months, using a fixed bicycle twice a day in a week (Three times) and 45 minutes each day with the severity and tolerance of the patients. The data were analyzed by SPSS 22 software, using one-way analysis of covariance.

Results: controlling the effect of pre-test, there was a significant difference between the mean post-test scores of cognitive abilities in the experimental and control groups, and the mean scores in the experimental group were higher than that of the control group (p<0.01: F1.11=10.186)

Conclusions: Conclusion Attending at the aerobic exercise program is effective in improving the cognitive abilities of patients with Alzheimer’s disease.
Aims: In this study, we aimed to evaluate to what extent the patients with DBS were able to use remote control devices during pandemics.

Methods: 135 patients with DBS were recruited from the Movement Disorder Clinic of Marmara University Hospital. All patients who gave verbal consent were reached by telephone and a questionnaire consisting of 10 questions regarding the usage of DBS remote control was conducted.

Results: Median age of patients was 58.7 (6 and 79). 40%(n:54) of all patients was female and 60%(n:81) was male. 54% patient's educational status was primary school graduation. DBS indication of patients was 73%(n=99) PD, 16%(n=22) distonia, 7%(n=9) ET, and 4%(n=5) other MD. 93%(126/135) of the patients were using a remote control device. We found that initial training for the usage of remote control was given to 95(74%) patients, repetitive training was given only to 16(13%) patients. Moreover, 64%(82/128) of patients had never used remote control, 11%(14/128) of patients used less than 3 times in total, 16%(21/128) used regularly, 7%(9/128) of patients used daily as they close the stimulation in the evening and open in the morning, and 2 patients used it when they had clinical deterioration due to DBS.

Conclusions: Although initial training was given to almost every MD, repeated training was given to very few patients. The absence of simplified, Turkish-explanatory videos, simplified diagrams for patients caused patients and their relatives to feel a lack of information on this subject and to hesitate to use the remote control.
IS THERE A NEED TO INCREASE THE DOSE OF DOPAMINERGIC DRUGS IN PARKINSON'S DISEASE DURING COVID-19?

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Aims: Implications of COVID-19 infection on Parkinson's disease, particularly worsening of motor and non-motor symptoms have been reported in several case series and observational studies. There is debate on the change of dopaminergic drug need during COVID-19. The theory has been proposed that dopamine synthetic pathway is possibly involved in the pathophysiology of COVID-19, as ACE2 and dopamine decarboxylase co-express and co-regulate in non-neuronal cell types, which may indicate dopamine depletion and the need for considering levodopa as treatment.

Methods: In this review summarized the impact of COVID-19 on Parkinson's disease symptoms and changes in levodopa daily dose requirements.

Results: Patients with Parkinson's disease may experience substantial worsening of motor and non-motor symptoms during COVID-19 and may need to increase the dose of dopaminergic drugs.

Conclusions: Clinicians should take pathophysiological changes of COVID-19 into consideration during adjusting therapy regimens in Parkinson's disease.
SUBCOMMISSURAL ORGAN-SPONDIN-DERIVED PEPTIDE PROMOTES RECOVERY OF NEURONAL FUNCTION AFTER HYPOXIA

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Embargo
THE ADDITIVE NEUROINFLAMMATORY AND NEUROIMMUNE EFFECT OF COMBINED SARS-COV-2 AND RESPIRATORY SYNCYTIAL VIRUS (RSV) CO-INFECTION IN A NOVEL AD5-HACE MOUSE MODEL

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Aims: Although it is known that patients with cerebrovascular comorbidities such as vascular dementia are more likely to have worsened post-acute neurologic sequelae after infection with the novel coronavirus SARS-CoV-2, the combined sequelae of other viral infection comorbidities remains unknown. Respiratory syncytial virus (RSV) is one of the most common respiratory viruses to infect children worldwide and has been increasingly recognized as a pathogenic infection in adults linked to neuroinflammation. We hypothesized that introduction of RSV in a novel Ad5-hACE2 mouse would potentially aggravate neurological damage, neuroinflammation, and engender a neuroimmune responses after SARS-CoV-2 infection.

Methods: Mice were oropharyngeally transduced with $1.5 \times 10^9$ plaque-forming units (PFU)/mouse of Ad5-hACE2 under Animal Biosafety Level 2 (ABSL2) conditions to induce ACE2 expression in lungs. Four days later mice were inoculated with either saline or SARS-CoV-2 with or without RSV via intranasal administration by the ABSL3-trained staff with a SARS-CoV-2 dose of $1 \times 10^5$ TCID$_{50}$/mouse and RSV ($2 \times 10^7$), respectively to induce viral infection.

Results: GFAP, and NeuN proteins in brains were analyzed by immunofluorescence to measure both immune reactivity and neuronal cell integrity. SARS-CoV-2 + RSV co-inoculations induced GFAP in corpus callosal region and NeuN positive neuronal dropout in cortex were observed, with trends indicating higher immunoreactivity and lower neuronal cell count in combined co-inoculates than single inoculate alone.

Conclusions: Additional studies elucidating the exact mechanism SARS-CoV-2-mediated cerebrovascular injury and its combined role in comorbid RSV infection sequelae may broaden therapeutic insight into the pathogenesis and treatment of both viruses.


Conclusions: COVID-19 and Alzheimer’s disease
Aims: ND0612 is a continuous, subcutaneous levodopa/carbidopa delivery system in development for patients with Parkinson's disease experiencing motor fluctuations. Trials of drug-device combinations typically require several hours of in-person trainings, support, and monitoring. We describe the challenges of conducting such studies under the extraordinary conditions imposed by the pandemic.

Methods: A COVID19-Taskforce was established to rapidly adapt study execution strategies and tactics, balancing patient safety with good study practice.

Results: A risk assessment was immediately performed leading to a protocol amendment of the BouNDless pivotal trial (NCT04006210). Almost 60% of onsite study-visits were given the option to be conducted virtually (with clear guidance); the rest (including screening) were required to continue in-person. Digital health/decentralized clinical trial techniques were implemented to address difficulties in conducting virtual visits in countries with limited network infrastructures/smartphone availability. Nurse-educator support visits at patient homes were partially replaced by virtual visits, and the nurse call-centre was extended to provide 24/7 patient support. Study supplies (including investigational product) were sent directly to patient’s homes, and sensors were added to collect objective motor measurements. Increased sponsor involvement (e.g. webinars and increased investigators support) improved communications. COVID19 testing was available for study monitors.

Conclusions: The changes implemented were well-accepted by the investigators and patients and ensured patient safety while maintaining the clinical trial integrity. We found that clear and frequent communication, with a balanced ‘hybrid’ mix of virtual and in-person approaches, successfully enabled the safe continuation of pivotal clinical trials with this drug-device combination in patients with PD.
COVID-19 PANDEMIC AND PARKINSON’S DISEASE: IMPACT ON PATIENT CARE AND DISEASE BURDEN IN GERMANY

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Aims: To assess the impact of the global COVID-19 pandemic on the medical care of patients with Parkinson’s disease (PD) in Germany in a nationwide online survey.

Methods: The ParCoPa (“PD in the COVID-19 pandemic”) study was conducted as a nationwide cross-sectional survey. From 12/2020 to 03/2021 patients with PD (n = 137) and their caregivers (n = 75) participated in the online survey. Health care professionals (n = 40) involved in PD care participated in a separate online survey about the impact of the pandemic on their work with PD patients.

Results: Worsening of PD symptoms was reported by 45.2% of the patients and 71.0% of the caregivers, while 82.5% of the physicians observed a symptomatic worsening of their PD patients. From the patients’ point of view, motor symptoms worsened the most (37.5%), followed by neuropsychiatric (29.7%) and sleep-associated (29.2%) symptoms. Negative impact on the quality of medical care was reported by 37.5% of the patients and 58.0% of the caregivers, while 97.5% of the physicians reported a negative impact on their PD patients’ medical care. Routine visits to neurologists occurred less frequently for 20.0% of the patients and most of the patients stopped entirely to participate in exercise (84.7%) and support groups (72.7%).

Conclusions: Our analysis raises awareness that medical care for PD patients is significantly compromised in the pandemic. Telemedicine approaches and measures to minimize the risk of infection during outpatient visits may be appropriate to adequately treat this vulnerable patient population in the context of this and future pandemic situations.