P-1. AGE PROGRESSION OF BEHAVIORAL ABNORMALITIES IN L61 αSyn MICE

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Objectives
The Thy-**1αSyn** (L61) mouse model for alpha-synuclein (a-syn) pathology has been reported to display an early behavioral phenotype and deposition of a-syn in brain. We aimed at characterizing the age progression in hind limb clasping and anxiety-like behavior in such mice.

Methods
Hind limb clasping score and open field test were analyzed in L61 and wt mice at 3, 6, 9 and 12 months of age (n>14/group) using two-way ANOVA and Mann-Whitney U test. Since males develop more aggressive phenotype, both sexes were included. The levels of alpha-synuclein will be analyzed with various immunoassays.

Results
Compared to wt, L61 mice displayed abnormal hind limb clasping. In male L61 mice there was a tendency for abnormal hind limb clasping already at 3 months and this difference became significant at 12 months. In open field test L61 mice manifested a hyperactive phenotype (increased velocity and distance moved) at 6 months. This phenotype progressed with age and was more pronounced in males. Moreover, L61 mice showed strong thigmotaxis (as they preferred to move close to the walls).

Conclusions
We found that the L61 transgenic mice develop age-progressive motor impairments (severe hind limb clasping) and display characteristic features of anxiety-like behavior. Ongoing analyses will demonstrate how the various behavioral features correspond to a-syn levels and extent of a-syn deposition in brain.

P-2. SHINING INFRARED LIGHT ON ALZHEIMER'S DISEASE

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Background
Oligomers of the amyloid-β peptide (A) are suspected to be the main toxic species involved in Alzheimer's disease. In spite of their relevance, there is no consensus regarding the structure of the oligomers. The A peptide has two main variants of different lengths (A40 and A42). Their relative abundance is decisive for the severity of the disease and mixed oligomers may contribute to the toxic species. However, little is know about the extent of mixing in oligomers.

Aims
(i) To assess whether and to what extent Aβ40 and Aβ42 co-aggregate, (ii) To reveal the internal structure of Aβ40 and Aβ42 in homo-oligomers.

Materials and Methods
Fourier transform infrared (IR) spectroscopy in combination with 13C-labeling and spectrum calculation to study oligomers of Aβ40, A42, and of Aβ40:Aβ42 mixtures.

Results
When 12C- and 13C-peptides are mixed, the IR spectrum changes in a characteristic way indicating incorporation of both Aβ40 and Aβ42 in common -sheets of Aβ40:Aβ42 hetero-oligomers [1]. Spectrum calculations revealed that the shift depends on the internal structure of the peptide molecules, i.e. whether they contribute just one strand to the -sheets, two adjacent strands, or three adjacent strands.

Interpretation
The hetero-oligomer results indicate a largely random distribution of Aβ40 and Aβ42 in the β-sheets of the mixed aggregates [1]. The homo-oligomer results are well explained by flat, antiparallel β-sheets with at least four β-strands or by β-barrels to which each peptide molecule contributes at least two adjacent strands [2].

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P-3. DOPAMINERGIC DYSFUNCTION IN THE (THY-1)-H[A30P] α-SYN TRANSGENIC MOUSE MODEL.

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Objectives
We have earlier shown that the (Thy-1)-h[A30P]α-syn transgenic mice display early fine motor impairment and age-related increase in α-syn pathology in the brain. In this study, the aim was to further study the mechanistic link between the dysfunction of the dopaminergic system and α-syn aggregation.

Methods
Tyrosine hydroxylase (TH) immunostainings were conducted on brain sections at 2, 5, 8 and 11 mo of age. We also performed oligomer-specific ELISA to assess the level α-syn oligomers in the midbrain homogenates. In addition, to assess the degree of misfolding and density of α-syn aggregates we employ PK resistant western blot assay. Furthermore, we plan to visualize the direct protein-protein interaction of α syn oligomerization in these mice tissue using PLA.

Results
Our preliminary results indicate a loss of TH immunoreactivity in the dopaminergic neuronal processes in the midbrain of α- syn transgenic mice at 8-month-old (mo) age when compared to age-matched controls. In addition, we saw significant increase in the levels of soluble α-syn oligomers in midbrain homogenates of 5 mo A30P mice when compared to 11 mo A30P mice. Furthermore, a trend towards high PK resistance to α-syn species in soluble midbrain homogenates of 8 & 11 mo mice was observed.

Conclusions
We found that the A30P mice displays characteristics of PD, such as decreased immunoreactivity of dopaminergic neurons in the midbrain. Ongoing analysis will discern the correlation between early changes in the dopaminergic pathway and α-syn dysregulation.

P-4. POORLY-CONTROLLED DIABETES INCREASES THE RISK OF COGNITIVE IMPAIRMENT AND ACCELERATES THE PROGRESSION TO DEMENTIA IN SWEDISH OLDER ADULTS

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Background
Despite the well-established link between diabetes and dementia risk, the impact of prediabetes and diabetes on the prodromal dementia phase remains controversial. In this study, we investigated whether prediabetes and diabetes increase the risk of cognitive impairment no dementia (CIND) and accelerate the progression from CIND to dementia.

Methods
In the Swedish National Study on Aging and Care-Kungsholmen (SNAC-K), one cohort of cognitively-intact individuals (n=1837) and one cohort of individuals with CIND (n=671) aged ≥60 years were followed for up to 15 years. At baseline and each follow-up (every 3 or 6 years), a neuropsychological test battery was administered, and the domains of episodic memory, processing speed, executive function, visuospatial abilities, and language were derived. CIND was defined as having no dementia and cognitive performance ≤1.5 SDs below age group-specific means in at least one cognitive domain. Dementia was diagnosed according to DSM-IV criteria. Diabetes was diagnosed on the basis of medical history and glycated hemoglobin (HbA1c) ≥6.5%. Prediabetes was identified as HbA1c 5.7-6.4% in diabetes-free participants. Data were analyzed with Cox regression models adjusted for possible confounders.

Results
At baseline, in the cognitively-intact cohort, 133 (7%) participants had diabetes and 615 (34%) had prediabetes. In the CIND cohort, 84 (13%) had diabetes and 238 (36%) prediabetes. During follow-up (mean 9.2 ± 3.0 years [range=2.2-15.5 years]), 544 (30%) individuals in the cognitively-intact cohort developed CIND. Poorly-controlled diabetes (HbA1c≥7.5%) was associated with 2-times higher risk of CIND (HR 2.0, 95% CI:1.11-3.48) than diabetes-free participants. In the CIND cohort, 132 (20%) individuals progressed to dementia during follow-up (mean 7.7 ± 4.0 years [range=0.2-15.2 years]). Poorly-controlled diabetes was associated with 3-times higher risk of dementia progression (HR 3.3, 95% CI: 1.29-8.33). No associations between prediabetes and CIND were detected in either cohort.

Conclusions
Poorly-controlled diabetes increases the risk of cognitive impairment and accelerates its progression to dementia.

P-5. G273R MUTATION CHANGES THE BINDING PROPERTIES OF TAU TO MICROTUBULES AND F-ACTIN

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The G273R mutant located in the first repeat region of the Microubule Associated Protein Tau (MAPT) was found in a patient diagnosed with Frontotemporal Dementia (FTD) [1]. The aim of this study was to characterize the properties of this mutant compared to its wild type counterpart. Tau is involved in the stabilizing of microtubules and microtubule interaction with F-actin[2]. Tau forms proteopathic aggregates in several diseases.

For in vitro studies, the protein was expressed and purified from E. coli. Monomeric tau was used in fibrillation studies and to determine the binding to microtubule and F-actin.

Fibrillation experiments of the tau variants show that the seeding efficiency is sequence dependent and fibrils formed from 0N4R G273R are thinner than 0N4R PWT tau (cysteine free pseudo wild type), but 0N3R G273R fibrils are thicker than 0N3R PWT. Binding studies reveal that the G273R mutant increases binding affinity to microtubules and decreases binding to F-actin for the 4R-Tau variant.

Our results of the G273R mutation with increased binding affinity to microtubules and decreased binding affinity to F-actin could be detrimental for the cell. The different thicknesses of G273R fibrils suggests another polymorph of the fibrils formed from the mutant.

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P-6. hIAPP AGGREGATION AND DITYROSINE GENERATION UPON FE(II) BINDING.

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An underlying cause for many neurodegenerative diseases are amyloid aggregates. Neurotoxic plaques in Alzhemier's disease (AD) are mainly composed of fibril aggregates of the Aβ peptide, while in diabetes mellitus type-2 the amyloid aggregates found in the b-cells predominantly consist of the peptide hormone islet amyloid polypeptide (IAPP). Previous studies have shown that amyloid proteins can seed aggregation of other peptides or proteins, which can explain the prevalence of amyloid diseases occurring together. Thus, it is important to identify common factors that influence the aggregation process of various amyloidogenic proteins or peptides. One such factor appears to be metal ions.

Here, we investigated how the metal ion Fe(II) binds to human IAPP and how such binding affects the hIAPP aggregation in vitro. The binding of Fe(II) was investigated in different conditions such as different pH levels, in membrane-mimicking SDS micelles and vesicles, all in a reducing environment to keep the metal ions as Fe(II). The C-terminus residue of hIAPP is tyrosine, which allowed us to monitor the generation of Fe(II)-induced dityrosine, which is linked to oxidative stress. The main methods used were fluorescence spectroscopy, ThT kinetics studies and TEM imaging. Our results show that hIAPP binds Fe(II) stronger at physiological pH than acidic, which suggests that histidine is involved in the Fe(II) binding, and that a membrane like environment weakens the binding affinity. Dityrosine is more readily produced in an oxidative environment, promoted by hydrogen peroxide, which is in accordance with previous studies. Overall, the binding of Fe(II) ions slowed down the hIAPP aggregation process.

P-7. OCCUPATIONAL COMPLEXITY AND FINGER COGNITIVE CHANGE

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Introduction
Within the multimodal lifestyle intervention for cognitive impairment known as FINGER we investigated if previous occupational complexity affects the yearly difference in cognitive change between the intervention and control group.

Methods
The FINGER study was a 24-month multidomain lifestyle intervention (diet, exercise, and cognitive training) RCT for cognitive impairment conducted in Finland between 2009 and 2014. This current study used 1026 participants from this study who were retired start of the study and had information about their latest occupation available. The main outcome of the study was the yearly difference in cognition (NTB Total, Executive function, Memory and Processing speed) between the active and control group.

Results
There was a significant effect of occupational complexity with data on the executive function outcome (ß[SE]: .03 (.01), p= .044). No other significant interactions were found for the yearly cognitive change difference. We also found a significant effect of previous occupational complexity on baseline cognition, this effect was present for all three types of complexities and for all five types of cognition even after controlling for education.

Discussion
The cognitive change in an RCT multimodal lifestyle intervention can be affected by previous occupational complexity. This facet of mental stimulation should be further investigated within RCTs for cognitive impairment. This study also found that occupational complexity has an effect on late-life cognition that cannot be explained by educational achievements alone. The effect of occupational complexity on late-life cognition may be achieved through both differential preservation and preserved differentiation.

P-8. LIFELONG COGNITIVE RESERVE REDUCES THE DEMENTIA RISK ASSOCIATED WITH DIABETES AND HELPS PRESERVE BRAIN VOLUME

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Introduction
Diabetes is a major risk factor for dementia and loss of brain integrity. Lifelong exposure to cognitively- and socially-enriching activities, that is cognitive reserve (CR), could buffer these risks. We investigated whether CR compensates for the risk of diabetes-associated dementia in relation to brain integrity.

Methods
A cohort of 2515 dementia-free older adults from the population-based Swedish National study on Aging and Care-Kungsholmen (SNAC-K) was followed over 15 years. Dementia was diagnosed according to standard criteria. Diabetes was ascertained through medical history, medication use, medical records, or glycated haemoglobin. Using structural equation modelling, CR was operationalized by education, work complexity, leisure activities and social network. At baseline, a subset of 407 participants underwent brain MRI scans; brain volumes were measured. Cox and linear regression models were used for analysis.

Results
During follow-up up (median=11.4 [interquartile range, 6.11–11.7] years), 362 participants developed dementia. People with diabetes had 50% increased risk of dementia than those without. Moderate and highest CR were associated with decreased dementia risk. Participants with diabetes and with low CR had increased hazard ratio (HR=2.19, 95%CI 1.34–3.57) of dementia. However, in participants with diabetes who had moderate-to-high CR, the risk of dementia was no longer statistically significant (HR=1.52, 95%CI 0.89–2.57). Participants with diabetes who had low CR had the smallest brain volumes. Nonetheless, participants with diabetes with moderate-to-high CR had similar brain volumes to those of diabetes-free people.

Discussion
High lifelong CR appears to counteract the risk of dementia associated with diabetes and its negative impact on the brain

P-9. CONCORDANT AND DISCORDANT AMYLOID CSF/PET BIOMARKERS IN ALZHEIMER'S DISEASE: A LONGITUDINAL INVESTIGATION

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Background
While Florbetapir-PET and CSF-Aß42 biomarkers are deemed interchangeable, mismatch between these measures has been reported in around 10-20% of cases. Here, we hypothesize that biomarker discordance is due to differences in amyloid processing and kinetics in the CSF vs. in the brain, whereby CSF represents an instantaneous measure of ongoing amyloid accumulation, while PET represent an integral measure of resulting amyloid accumulation. We predict that the earliest stage of amyloid deposition consists of isolated CSF+/PET- cases only.

Method
We retrospectively selected N=867 cases from the Alzheimer's Disease Neuroimaging Initiative (ADNI), ranging from cognitively normal to overtly demented, with Florbetapir-PET and CSF-Aß42 measurements obtained within 3 months. We additionally collected available longitudinal PET/CSF follow-up data at 2-year follow-up (N=289). We investigated longitudinal changes in amyloid biomarkers abnormalities (PET and CSF) to test our prediction that the earliest stage of amyloid deposition consists of isolated CSF+/PET- cases only.

Result
Longitudinal assessment showed that, at 2-year follow-up, 7.9% of concordant negative cases progressed towards isolated CSF positivity. This pattern of progression was three time more likely than direct progression to concordant positive biomarkers. 29.4% of subjects with isolated CSF positivity progressed to full biomarker positivity at 2-year follow-up (compared to only 2.6% progressors among fully negative cases). Contrarily to our hypothesis, however, 2.6% of concordant negative cases followed an alternative pathway, with PET becoming positive first. 21.4% of subjects with isolated PET positivity progressed to full biomarker positivity at 2-year follow-up (compared to only 2.6% progressors among fully negative cases)

Conclusion
Our results suggest that different trajectories towards abnormal amyloid biomarkers are possible, with either CSF or PET becoming abnormal first. The hypothesis whereby CSF represents a dynamic measure of ongoing amyloid accumulation may only partially account for biomarkers mismatches. This advocates for the use of diverse biomarkers for patient stratification.

P-10. EXPLORING AUTOANTIBODY REPERTOIRES WITHIN ALZHEIMER'S DISEASE

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Aims

Despite decades of intense research, the cause of AD remains unknown. Recently, there has been a focus on the inflammatory components of AD. There is an extensive activation of the immune system within the CNS of AD patients, but neither its cause nor its role in AD is known. However, there are strong indications that the inflammation has an autoimmune character. Considering this, there is an imperative need to examine autoimmunity within AD. We used a proteomic approach to determine the autoantibody profiles within plasma and cerebrospinal fluid (CSF) within AD patients and healthy controls.

Methods

Paired plasma and CSF samples from 23 healthy controls and 49 patients were used. In addition, 2 non-paired patient plasma samples and 18 non-paired patient CSF samples from were included. One 380-plex and one 314-plex targeted suspension bead array (SBA), consisting of microspheres with immobilized antigens, were used to analyze autoantibody profiles in all samples.

Results

The resulting data revealed an increased autoantibody response towards antigens SLC17A6, MAP1A, and MAP2 in patients compared to healthy controls. Furthermore, the paired CSF and plasma samples were used to investigate the correlation of autoantibody profiles within patients. The correlation was found to follow a normal distribution, with correlation being higher in antigens displaying stronger autoantibody reactivity.

Conclusions

As the found antigens have displayed wide reactivities in previous, unpublished studies, further investigation is required to determine their role in AD. This work represents one of the first large-scale studies on the correlation of autoantibody profiles in plasma and CSF.

P-11. ALTERATIONS IN FREE FATTY ACIDS AND PHOSPHOLIPIDS IN AN APP KNOCK-IN MOUSE MODEL FOR ALZHEIMER'S DISEASE

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Background
Alzheimer's disease (AD) is a neurodegenerative disorder and inflammation is part of the neuropathology in AD. Resolution of inflammation is orchestrated by specialized pro-resolving mediators derived from omega-3 (DHA, EPA) and -6 fatty acids (AA). Brain is the richest organ in lipid content. Phospholipids are biologically crucial structures for building double layer cell membranes. Studies on membrane lipid composition of AD patients have shown alterations in lipid composition. Different regions of the brain differ in phospholipid composition and determination of regional phospholipid distribution can be achieved for clarifying the role of phospholipids in the brain. To understand the role of dysfunction of beneficial lipids in AD, we investigate the correlation between lipid composition and AD neuropathology using an App knock-in AD mouse model which harbours high Aβ42 levels and exhibits neuroinflammation.

Methods
This exploratory study involves characterization of bioactive lipid mediators and investigating their stereochemistry in hippocampus, cortex, cerebellum and liver from the App knock-in and wild-type mice using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and matrix-assisted laser desorption-ionization-imaging mass spectrometry (MALDI).

Results
Positively charged lipid ion species were abundantly detected and the distribution pattern of lipids between WT and APP knock-in was similar. CA1, CA2, CA3 and CA4 regions, and DG show changes in lipid expression with aging. The lipids that were increased m/z 800.6 (PC 34:0 K+), 882.5 (PC 34:1 K+), 846.4 (PC 38:5 K+), 844.6 (PC 38:6 K+) at 18 months of age in both APP knock-in and wildtype compared to 2 and 8 months of age. There was an increase in grey matter with m/z 828.4 (PC 36:0 K+) at 8 months of age in APP knock-in.

Conclusion
Our data show differential distribution of phospholipids within the brain of the App knock-in mice, and that the levels change with age between these mice and WT mice.

P-57. THE ROLE OF ASTROCYTES IN ALZHEIMER'S DISEASE – FOCUS ON CHOLESTEROL AND Aβ DEPOSITS

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Although Alzheimer's disease (AD) is the most common cause of dementia in the elderly, the molecular and cellular mechanisms behind the disease remain unclear. The key neuropathological hallmarks of AD are amyloid plaques, mainly consisting of aggregated amyloid beta (Aβ), intracellular neurofibrillary tangles, composed of hyperphosphorylated tau and chronic neuroinflammation. The Aβ protein is very aggregation prone and forms soluble aggregates, which aggregate further to insoluble fibrils and deposit as plaques. For a long time it was believed that the plaques were the toxic feature in AD, but in recent years the focus has been shifting towards the soluble aggregates of Aβ, since there is no clear correlation between the number of plaques and the severity of the disease.

Accumulating evidence indicates that astrocytes can play a central role during AD progression. Our group has previously demonstrated that astrocytes ingest large amounts of aggregated Aβ, but then store, rather than degrade the ingested material. The incomplete digestion results in a high intracellular load of neurotoxic Aβ species, lysosomal dysfunction and secretion of extracellular vesicles (EVs), carrying N-truncated Aβ. In addition to Aβ, the EVs secreted from astrocytes exposed to Aβ protofibrils contain higher levels of ApoE than EVs from untreated astrocytes.

Cholesterol has been suggested to play a role in AD development, but the mechanism behind this link is still unclear. Brain cholesterol is synthesized by astrocytes and oligodendrocytes and is almost completely isolated from other pools in the body. The aim with the present study was to investigate if Aβ accumulation in astrocytes affect their cholesterol homeostasis. Interestingly, we found that the cholesterol expression pattern in astrocytes was changed following Aβ exposure. While the cholesterol in control astrocytes was evenly distributed in the cells, cholesterol in Aβ exposed astrocytes was mainly concentrated around the Aβ inclusions.

P-13. JOINT TRAJECTORIES OF EPISODIC MEMORY AND ODOR IDENTIFICATION IN OLDER ADULTS: PATTERNS AND DETERMINANTS

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Aims
Emerging evidence has shown that olfactory function is closely linked to memory function. The aims of this study was to assess whether olfactory function and episodic memory function follow similar trajectories, to identify different patterns of joint trajectories of the functions, and to detect determinants of the patterns.

Methods
Using data from the Rush Memory and Aging Project, 1041 dementia-free participants were identified at baseline and followed for up to 8 years with annual assessments for episodic memory (composite of 7 tests) and odor identification (Brief Smell Identification Test). Data on demographics, medical conditions, social and lifestyle factors were collected by self-report or medical examination. Trajectories of episodic memory and odor function were first modeled individually and then jointly over time using growth mixture models to identify latent classes (patterns) of the joint trajectories. Multivariate logistic regression was used to identify predictors of the patterns.

Results
Both episodic memory and olfactory function showed similar trends over the follow-up time. Three distinct patterns of joint trajectories in both functions were identified; 1) Class 1-stable average performance in both function (n=700, 62.2%), 2) Class 2- stable average episodic memory and declining odor identification (n=237, 22.8%), and 3) Class 3- decline in both functions (n= 104, 10.0%). Compared to the Class 1, people in Class 2 were more likely to be older, male, and to be less socially active. People in Class 3 were more likely to be older, less educated, APOE ϵ4 carriers, to have depression and lower late life cognitive activity. ϵ

Conclusion
Episodic memory and odor function show similar trajectories in aging, which can be jointly characterized as stable, odor decline only and joint decline. Age, education, APOE ϵ4, depression, and leisure activities may be predictors for decline in both functions.

P-14. Pd(II) ION BINDING TO THE AMYLOID-BETA (Aβ) PEPTIDE: RESIDUE-SPECIFIC INTERACTIONS RETARD Aβ AGGREGATION

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Brain deposits of insoluble amyloid plaques consisting mainly of aggregated amyloid-β (Aβ) peptides are a hallmark of Alzheimer's disease (AD), the most common cause of dementia worldwide. A binding site for metal ions is present in the N-terminal Aβ segment, and the AD brain plaques are known to contain elevated levels of transition metals such as Cu, Fe, and Zn. Even though AD patients display altered metal homeostasis, the details of the metal chemistry involved in AD disease pathology remain unresolved. Here, we use nuclear magnetic resonance (NMR) and fluorescence spectroscopy together with atomic force microscopy (AFM) imaging to show that Pd(II) ions display specific binding to the N-terminal Aβ segment, and that such binding retards the Aβ aggregation process and directs it towards non-fibrillar aggregates. The His6, His13, and His14 residues are implicated as binding ligands, and the Pd(II)/Aβ binding affinity is around 200 μM.

P-15. TETRAZINE-FUNCTIONALIZED CLEARING AGENT FOR SAME DAY IMMUNO-PET IMAGING

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Molecular imaging with positron emission tomography (PET) has become an important diagnostic tool in the recent years. Plaques in the brain of Alzheimer's disease (AD) patients can already be visualised. Traditionally, radioligands are small, lipophilic molecules with fairly fast pharmacokinetics. Antibodies are highly specific for their target and therefore an attractive alternative to small molecules. Depending on the modification and doses administered, antibodies engineered to enter the brain may display brain concentrations up to 80-fold higher compared with unmodified antibodies, reaching similar brain concentrations as those observed with small, lipophilic radioligands. However, compared to small molecules, antibody based ligands have a long biological half-life in blood and are therefore usually radiolabelled with long-lived PET radionuclides such as iodine-124 (124I; half-life 4.2 days) or zirconium-89 (89Zr; half-life 3.3 days). PET with long-lived radioisotopes is associated with high absorbed radiation dose for patients and complicated logistics since the patient does not receive the tracer dose on the same day as the PET examination.

This project aims to facilitate antibody-based PET imaging with short-lived PET radionuclides to allow PET imaging on the same day as injection and more favourable dosimetry. In a proof of concept study, single photon emission computed tomography (SPECT) was used to visualize brain retention and biodistribution of the Aεantibody mAb158, radiolabelled with iodine-125 and modified with TCO. Using bio-orthogonal chemistry, more specifically the Diels-Adler click reaction, a tetrazine-functionalized clearing agent (CA), which reacts quickly with the TCO-modified antibody, was injected to provide a faster radioligand clearance from blood. SPECT images showed immediate liver accumulation upon CA administration, providing evidence that the method works as intended and a good foundation for further studies.

P-16. PREPARATION AND CHARACTERIZATION OF Aβ42 OLIGOMERS

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Oligomers of the amyloid beta peptide (Aβ) are considered the most neurotoxic aggregates of the peptide (Haas et al, Nat Rev Mol Cell Biol, 2007). Studies of Aβ oligomers is complicated by their intrinsic metastability and heterogeneity. Certain protocols have been developed to stabilize the oligomers and make it possible to study them with biochemical and biophysical techniques.

Fourier Transform InfraRed (FTIR) spectroscopy is a biophysical technique which measures absorption of infrared light by matter and is sensitive to stretches and bends of chemical bonds in the molecules. The technique can be used to determine the secondary structure of the proteins and therefore is a powerful tool to study protein misfolding and aggregation (Barth, Biochim Biophys Acta, 2007).

The aim of the current study is to optimize the protocols for Aβ oligomer preparation, as well as characterize them with a combination of gel electrophoresis and FTIR spectroscopy methods.

To prepare Aβ42 oligomers, monomeric solutions of the peptide were incubated with submicellar concentrations of Sodium Dodecyl Sulfate (Barghorn et al, J Neurochem, 2005) or Dodecyl PhosphoCholine (Serra-Batiste et al, Proc Natl Acad Sci USA, 2016) at physiological conditions. The oligomeric solutions were studied by native and SDS-PAGE electrophoresis, as well as FTIR spectroscopy.

Aβ42 oligomers of 18/22 and 36/54 kD were produced after incubation of the peptide in 0.2 % and 0.05% SDS concentrations, respectively. DPC treatment led to formation of oligomers resolving around 18 kD on SDS-denaturing gel. IR spectra were specific for the prepared oligomeric solutions. Spectra for detergent induced oligomers were clearly distinct from those for oligomers obtained in the absence of any detergents. IR spectra were in agreement with anti-parallel β-sheet conformation.

P-17. DEVELOPMENT OF A NOVEL SPECIES-INDEPENDENT TfR-BINDING ANTIBODY

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Crossing the blood brain barrier (BBB) is a major challenge when developing diagnostic positron emission tomography (PET) radioligands that target pathology inside the central nervous system (CNS). Antibodies are good candidates for diagnostic markers because they can target disease pathology in the brain very specifically. However, antibodies are large and lipophobic, and thus, only very small amounts pass the BBB.

Antibodies engineered to create a "molecular Trojan Horse" can bypass the BBB. One such Trojan horse strategy is to target the transferrin receptor (TfR) present on BBB endothelial cells. TfR carries bispecific antibodies (targeting both TfR and pathology in the CNS) across the BBB in endosomes and releases them into the brain. However, this strategy to create antibody based PET markers for pathology in the CNS has so far only been demonstrated in mice.

Currently, there are no species-cross-reactive TfR-binders. The aim of this project is to develop a new antibody that binds to both mouse and human TfR, which would improve translation from bench to clinic of diagnostic antibody-based techniques for neurodegenerative diseases.

Here, we have produced Fab fragments of three mouse and human TfR-binding clones. We have studied the affinity of these Fab fragments in vitro with Octet. We also radiolabeled the Fab fragments and injected them intravenously into mice to determine the brain penetrance and the brain-to-blood concentration ratio.

Although all three Fab fragments still bind to human and mouse TfR, none of them entered the brain after intravenous injection. Next, we will investigate whether the radiolabeling process has an effect on the Fab fragment's affinity for mouse TfR.

P-18. BRAIN CHANGES AND FAST DECLINE IN COGNITION AND GAIT SPEED: FINDINGS FROM THE SNACK-MRI STUDY

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Background and aims
People with cognitive and physical decline are at higher risk of dementia, but no studies have been conducted on the neuroimaging signature of the joint decline in cognition and physical function. We aimed to test the association between brain magnetic resonance imaging (MRI) volumes and lesions – and their change over time – in individuals with cognitive and gait speed decline.

Methods
A sample of 385 participants was derived from the Swedish National Study on Aging and Care in Kungsholmen. Brain MRI markers included volumes of total brain tissue, hippocampus (HV), lateral ventricles and white matter hyperintensities (WMH). Cognition was assessed through the Mini Mental State Examination (MMSE) and physical function through gait speed (m/s). Based on the decline pattern over 12 years, estimated with linear mixed models, participants were divided into four groups: non-decliners (reference group), fast decliners only in cognition, fast decliners only in gait speed and fast decliners in both cognition and gait speed. Multinomial logistic regression was used to test the association between baseline brain data and the speed of decline over time. Linear mixed models were used to estimate the association between changes in brain MRI measures and the speed of decline in cognition and gait speed.

Results
A smaller total brain tissue volume (p=.002) and HV (p=.013), and a greater load of WMH (p=.015) and ventricles volumes (p<.001) were associated with a faster decline in both cognition and physical function as compared with non-decliners. We observed a greater loss in the total brain tissue volume (β:-12.1;95%CI:-18.1;-6.0), a smaller HV (β:-0.13;95%CI:-0.17;-0.08), a greater accumulation of WMH (β:1.54;95%-CI:0.48;2.59) and greater ventricular volumes (β:2.07;95%CI:0.70;3.43), as compared to non-decliners.

Conclusions
Smaller brain volumes and more lesions, together with loss of neural integrity over time, predicts faster and simultaneous decline in cognition and gait speed.

P-19. THE PROTEOME OF THE DENTATE TERMINAL ZONE OF THE PERFORANT PATH INDICATES PRESYNAPTIC IMPAIRMENT IN ALZHEIMER DISEASE

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Synaptic dysfunction is an early pathogenic event in Alzheimer disease (AD) that contributes to network disturbances and cognitive decline. Some synapses are more vulnerable than others, including the synapses of the perforant path, which provides the main excitatory input to the hippocampus. To elucidate the molecular mechanisms underlying the dysfunction of these synapses, we performed an explorative proteomic study of the dentate terminal zone of the perforant path. The outer two thirds of the molecular layer of the dentate gyrus, where the perforant path synapses are located, was microdissected from five subjects with AD and five non-demented controls. The microdissected tissues were dissolved and digested by trypsin. Peptides from each sample were labelled with different isobaric tags, pooled together and pre-fractionated into 72 fractions by high-resolution isoelectric focusing. Each fraction was then analyzed by liquid chromatography-mass spectrometry. We quantified the relative expression levels of 7322 proteins, whereof 724 showed significantly altered levels in AD. Our comprehensive data analysis using enrichment and pathway analyses strongly indicated that presynaptic signaling, particularly processes like exocytosis and synaptic vesicle cycle, is severely disturbed in this area in AD, while postsynaptic proteins remained unchanged. Among the significantly altered proteins, we selected three of the most downregulated presynaptic proteins; complexin-1, complexin-2 and synaptogyrin-1, for further validation, using a new cohort consisting of six AD and eight control cases. Semi-quantitative analysis of immunohistochemical staining confirmed decreased levels of complexin-1 and synaptogyrin-1 in the outer two-thirds of the molecular layer of the dentate gyrus in AD. Our in-depth proteomic analysis provides extensive knowledge on the potential molecular mechanism underlying synaptic dysfunction related to AD and supports that presynaptic alterations are more important than postsynaptic changes in early stages of the disease. The specific presynaptic proteins identified could potentially be targeted to halt synaptic dysfunction in AD.

P-20. CAN PERFORMANCE IN OLFACTORY FUNCTION PREDICT CONVERSION TO ALZHEIMER'S DISEASE? – A SYSTEMATIC REVIEW

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Deficits in the sense of smell, olfaction, are a common feature of Alzheimer's disease (AD) and might likely develop prior to the manifestation of memory symptoms. In recent years, a growing body of prospective studies has studied associations between baseline function in olfactory tests and later conversion to AD, both in large-scale population-based samples of older participants as well as in patient-groups who had already developed MCI at baseline.

The objective of the present systematic review was to synthesize all past longitudinal, prospective studies investigating olfactory testing in different olfactory domains as a method of predicting conversion to AD. In accordance with PRISMA guidelines, PubMed and Scopus, EMBASE, ISI Web of Science, PsycINFO were searched to determine the quantity of longitudinal research on this topic. Four prospective studies, predicting direct conversion to AD in a total sample of 7620 population-based older adults over an average follow-up time-span of 94 months, were included in the review.

The studies consistently found performance in olfactory identification to emerge as a significant predictor of later progression to AD. In addition, three prospective studies, predicting progression from MCI to AD in a total sample of 430 older adults were included. All studies found significant associations between olfactory performance and progression from MCI to AD. Due to heterogeneity regarding the olfactory identification test that was used in the studies, a conduction of meta-analysis was not feasible. In future, guidelines for presentation of statistical effects, based for example on standardized definitions of olfactory measures, might facilitate quantitative analyses of the predictive utility of olfactory function for AD.

P-21. A HIGHER FAT:CARBOHYDRATE RATIO IN DIET IS ASSOCIATED WITH BETTER COGNITIVE PERFORMANCE: BASELINE DATA FROM THE FINGER TRIAL

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Objective
The goal of this study was to investigate the associations between the dietary fat:carbohydrate-ratio (FCr) and cognition, using data from the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER), which was the first double blind randomized controlled trial to show that a multi-domain lifestyle intervention can improve cognition after two years in an elderly population at risk of dementia.

Method
Data were available from FINGER participants (N=1260, age 60-77). This cross-sectional analysis is based on baseline data collected before the randomization. Diet was assessed through a 3-day food record. The standardized log-FCr (calculated on energy percent) was used as the independent variable in a linear regression model, adjusted for age, sex, total energy intake, study center, BMI, cholesterol lowering medication, diabetes, smoking and apoE ϵ4-allele. Cognition was assessed with a neuropsychological test battery (NTB) composite score, including memory, executive function and processing speed sub-domains

Results
In this exploratory study, the median FCr was 0.69, with percentile 1-99 in the range 0.33-1.58. The log-FCr was significantly associated with the total cognitive composite score (β =0.033; 95% CI 0.008 • 0.063; p=0.024), and the executive function subdomain (β=0.049; CI 0.015 • 0.084; p=0.005). Associations for memory (β =0.022; CI -0.014 • 0.057; p=0.23), and processing speed (β =0.029; CI -0.014 • 0.072; p=0.19) were not significant. Intake of protein and the ratio saturated:unsaturated fat was not associated with cognition, and did not change the results when added to the model.

Conclusions
A higher dietary fat:carbohydrate ratio was associated with better performance on the cognitive composite score, and in the executive function subdomain. Our observations do not provide conclusions on causality, but indicate that further studies on associations and mechanisms linking FCr to brain health are warranted, as these may be important to consider in future dementia prevention initiatives.

P-22. QUANTIFICATION OF KETOSIS AFTER INTAKE OF DIFFERENT FATTY ACIDS WITHIN A 16-HOUR NON-CARBOHYDRATE WINDOW: A PHYSIOLOGICAL STUDY IN HEALTHY OLDER ADULTS

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Objective
The metabolic state ketosis is increasingly investigated in relation to brain health. Caprylic acid (C8), which constitutes 8% of coconut oil (Co), is known to be ketogenic. Our aim was to study how intake of Co and C8 interact with fasting ketosis. Sunflower oil (Su) was used as control, expected to not break fasting ketosis, although not being ketogenic in itself.

Method
Fifteen healthy older adults were tested in a within-subjects design in six different arms. After a 12-hour fast, the ketone body β-hydroxybutyrate was measured at 12 timepoints during 4 hours, after intake of coffee with cream (15 g) in combination with the test ingredients in a randomized order: 1. Su (30 g); 2. C8 (20 g) + Su (10 g); 3. C8 (20 g) + Su (10 g) + Glucose (50 g); 4. Co (30 g); 5. Co (30 g) + Glucose (50 g); 6. Co (30 g) + C8 (20 g). Questionnaires on hunger and tolerance of the drink were administered. Area under the curve for β-hydroxybutyrate was calculated, and then divided by time to report mean levels. ANOVA for repeated measures was used to compare arms.

Results
Mean levels of β-hydroxybutyrate (mmol/L) were in descending order 0.45 (arm 6 & 2), 0.28 (3), 0.22 (4), 0.17 (1), 0.08 (5). The difference was significant for arm 2 & 6 vs. 1, 3, 4, 5, and 5 vs. 3
& 4. Tolerance was good, and satiety was sufficient for all but 1-2 participants per arm.

Conclusions
In line with previous findings, we observed that C8—but not coconut oil—is ketogenic even with carbohydrate intake. However, in the context of a non-carbohydrate window, coconut oil may provide satiety to extend fasting ketosis. We also found that ketosis in the C8-arms was reduced after glucose intake.

P-23. CORRELATIONS OF NEURONAL CSF PROTEINS TO TAU PATHOLOGY AND BRAIN ATROPHY

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Although the hallmarks of Alzheimer's disease (AD) have been known for over a century, their role in disease pathology is still not completely understood. The aim of our study was to investigate how levels of CSF proteins correlate with Abeta and tau pathology to potentially shed new light on the pathological processes in AD.

We have profiled 230 proteins in CSF from two independent sample cohorts. One was a disease-focused cohort with 221 individuals including patients both with diagnosis and preclinical forms of disease. The other was a population-based study of 312 cognitively healthy participants at age 70. CSF Ab42, t-tau and p-tau concentrations were determined for all subjects and used to stratify also the population-based cohort into preclinical AD patients and controls. All samples were analysed using a bead-based antibody array with 313 antibodies and direct labelling of samples.

We found 35 proteins with moderate to strong correlation (Spearman's rho > 0.6) to levels of t-tau and p-tau in both the preclinical AD group and controls. These proteins were mostly neuronal and more specifically synaptic. In addition, 25 proteins showed weak correlations (Spearman's rho > 0.3) to levels of Ab42 in healthy individuals but, interestingly enough, no correlation in the preclinical AD group. Current analysis is ongoing to evaluate the associations of protein levels to specific tau fragments and brain atrophy as well as to further investigate these proteins in additional cohorts with in total over 400 individuals.

Our study provides a unique insight into how the CSF proteome varies in relation to AD pathology and can help to increase the understanding of early initiation and progression of this disease.

P-24. THE VIRAL PROTEIN CORONA DIRECTS VIRAL PATHOGENESIS AND AMYLOID NUCLEATION

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Viruses rely on the intracellular host machinery for replication, production of viral proteins and assembly. However, outside cells, as nanosized obligate intracellular pathogens, viruses share many biophysical properties with nanoparticles. Based on this biophysical equivalence, we hypothesized that viruses accumulate a host-derived protein corona layer in extracellular environments similar to nanoparticles. Here we show that respiratory syncytial virus (RSV) and herpes simplex virus 1 (HSV-1) accumulate rich and distinctive protein coronae in different biological fluids including human plasma, human bronchoalveolar lavage fluid, non-human primate plasma, and fetal bovine serum. Moreover, we show that corona pre-coating differentially affects viral infectivity and immune cell activation.

Additionally, we demonstrate that viruses can bind amyloidogenic peptides in their corona and catalyze amyloid formation via surface-assisted heterogeneous nucleation.

Importantly, we show that HSV-1 catalyzes the nucleation and accumulation of the amyloid-beta (Aβ42) peptide, which is the major constituent of amyloid plaques in Alzheimer's disease, in-vitro and in-vivo in Alzheimer's disease animal models.

Our results provide a proof-of-concept for the presence of an extensive and dynamic viral protein corona layer that is critical for viral-host interactions. Unlike the viral genome-coded surface proteins, the viral protein corona is an acquired structural layer that is dependent on the viral microenvironment resulting in different viral identities based on the target tissue and the target organism.

Additionally, the demonstration of corona-driven heterogeneous nucleation of amyloids illustrates convergence between viral and amyloid pathologies suggesting a direct physical mechanistic link that warrants further investigation.

P-25. CRISPR/CAS9-MEDIATED ALLELE SPECIFIC DISRUPTION OF PSEN1 CARRYING THE M146L MUTATION

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Objectives
Mutations in the presenilin 1 (PSEN1) gene cause early-onset forms of familial Alzheimer's disease (AD). Carriers of the PSEN1M146L mutation display increased generation of the more aggregation-prone amyloid beta form with 42 amino acids (Abeta42). These alterations are evident not only in the brain but also in peripheral cells, such as fibroblasts. Our objective was to evaluate if the CRISPR/Cas9 system can effectively disrupt the mutation-carrying allele whilst leaving the wild-type allele intact in AD fibroblasts and iPSC-derived neurons, thereby restoring the physiological cellular ratio of Abeta42/Abeta40.

Methods
Human fibroblasts from AD patients heterozygous for the PSEN1M146L mutation were transfected via nucleofection with a plasmid expressing PSEN1M146L gRNA and Cas9 protein. ELISA was used to measure Abeta40 and Abeta42 levels. Editing efficiency was assessed through Sanger sequencing and the Inference of CRISPR Editing (ICE) software. Human iPSC-derived neurons from the same patients will be transfected and assessed in the same manner.

Results
Through ICE we found robust indel formation in the DNA from gRNA/Cas9 treated human PSEN1M146L fibroblasts, whereas no indels were formed upon treatment with control vectors. Ongoing analyses will show whether the genetic manipulation in the targeted fibroblasts is paralleled by a reduction in Abeta42/Abeta40 ratio and if similar effects can be seen in iPSC-derived neurons.

Conclusions
We could demonstrate a selective disruption of the mutated PSEN1M146L allele in human AD patient fibroblasts using the CRISPR/Cas9 system. We believe that this system has the potential to be developed as a tool for future gene therapy against certain forms of familial AD.

P-26. FULLY BAYESIAN LONGITUDINAL UNSUPERVISED LEARNING FOR THE ASSESSMENT AND VISUALIZATION OF AD HETEROGENEITY AND PROGRESSION

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Longitudinal measurements of brain atrophy have revolutionized our understanding of how Alzheimer's disease (AD) pathophysiology evolves during the disease course. The distribution of neurofibrillary tangles (NFTs) in AD, varies across patients and it correlates with atrophy measures (in vivo) and specific profiles of cognitive loss.

The aim of this study is to construct and evaluate a longitudinal clustering experimental design that incorporates the following: 1) simultaneous clustering of longitudinal multivariate neuroimaging measures, 2) information of individuals with irregularly sampled observations (different sampling times), 3) comparison of the clusters with a control group, 4) study and fixation of potential confounding effects, 5) visualization of the resulting clusters for interpretation. This method was applied to the cortical thickness and subcortical volume measures of a sample of 72 AD patients and 31 cognitively unimpaired (CU) from the Alzheimer's Disease Neuroimaging Initiative. These measures were obtained by applying the FreeSurfer preprocessing pipeline to the T1-weighted images of the previous subjects acquired over a period of 2 years

We found 6 distinct patterns of longitudinal brain atrophy in AD patients. These patterns of atrophy included 3 typical AD diffuse patterns (Diffuse 1 (n=15), Diffuse 2 (N=15), Diffuse 3 (n=4)) and 3 atypical AD patterns: Minimal atrophy (n=23), hippocampal sparing early onset (n=4) and hippocampal sparing late onset (n=5). The clusters of patients differed not only in regional distributions of atrophy at baseline, but also in atrophy progression over time, age at AD onset, cognitive deficits at baseline and cognitive decline over time.

A framework for the longitudinal assessment of variability in cohorts with many neuroimaging biomarkers was successfully developed and the results show that it can be used to understand heterogeneity in the context of AD.

P-27. THE DRUG EDARAVONE INDUCES RAPID AGGREGATION OF AMYLOID-BETA PEPTIDES

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Aggregation of amyloid-beta (Aβ) peptides into oligomers that likely are neurotoxic is considered to be the main molecular cause of Alzheimer's disease (AD). Previous studies have shown that Edaravone, an antioxidant drug approved for treatment of various conditions including amyotrophic lateral sclerosis (ALS), show beneficial effects in cell and animal models exposed to Aβ-induced neurotoxicity. The molecular mechanisms of Edaravone are not fully understood, but one study reported that Edaravone inhibited Aβ aggregation and dissolved pre-formed Aβ fibrils in vitro. Here, we used atomic force microscopy (AFM) imaging and Thioflavin-T (ThT) fluorescence kinetic studies to show that Edaravone in fact induces rapid aggregation of Aβ peptides. We suggest that the beneficial effects of Edaravone on Aβ-induced neurotoxicity are caused by the drug promoting aggregation of toxic Aβ oligomers into larger non-toxic aggregates.

P-28. THE IMPACT OF LRRK2 ON THE UPTAKE AND ACCUMULATION OF AGGREGATED ALPHA-SYNUCLEIN IN ASTROCYTES

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Mutations in the LRRK2 gene represent one of the most common causes of familial Parkinson's disease (PD). LRRK2 is expressed in multiple cell types of the central nervous system, including astrocytes, and has been shown to play an important role in the endo-lysosomal pathway. The aim of this study is to clarify the impact of LRRK2 on the uptake and clearance of aggregated alpha-synuclein in astrocytes. As the most numerous glial cell type in the central nervous system, astrocytes have a great impact on the brain environment and might constitute a very potent treatment target. Astrocyte cultures derived from LRRK2 knock-out and wild-type mice were exposed to synthetic, sonicated alpha-synuclein pre-formed fibrils (PFFs). Alpha synuclein PFF uptake, accumulation and degradation was investigated by immunocytochemistry, ELISA, time-lapse microscopy and Western blot analysis. Additionally, astrocyte cultures from wild-type mice exposed to LRRK2 inhibitors were also investigated. Our results demonstrate that wild-type astrocytes readily internalize alpha-synuclein PFFs and only partially degrade the ingested material via the lysosomal pathway. The incomplete degradation leads to a high intracellular load of alpha-synuclein, resulting in mitochondrial abnormalities and spreading of alpha-synuclein to neighbouring cells. First results show an enhanced uptake and degradation of alpha-synuclein in LRRK2-deficient cells. LRRK2 is considered an attractive therapeutic target in PD and the role of LRRK2 for astrocytic uptake and accumulation of alpha-synuclein is therefore of high interest.

P-29. TOWARDS A BETTER UNDERSTANDING IN DIFFERENTIATION AND MIGRATION OF CELLS; A CLOSER STUDY IN VANGL2 SIGNALING

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Background
We had earlier shown that Wnt7a which is a ligand for Vangl2 receptor effects apoptosis and migration of epithelial cells.The purpose of current study is to investigate if Vangl2 is effected in wound healing and if this signaling pathway is effected when anesthetics are used.

Aims
1.Analyzing closer which molecules mediate Vangl2 signalings effect on apoptosis by investigating caspase-8 singling and investigate if this signaling pathway is intervened when anesthetics are applied.
2.Analyzing closer which molecules mediate Vangl2 signalings effect on migration.

Methods
Studies were done in HEK 293 cells, HeLa-cells, C2C12 cells as well as breast cancer cells and to a certain point in C17.2 cells.Cells were cultured on coverslips in 6-well plates until they became 80% confluent. They were transfected with a commercially bought Vangl2-GFP construct for 24 or 48 hours while the controls were transfected with GFP. Gene silencing studies will be done bye CRISPR/ CAS9.We further plan to simulate our studies with the help of bioinformatic programs and artificial intelligence.

Conclusion
We conclude that Vangl2 overexpression effects apoptosis and hypothezise that this is possibly done via caspase-8 signaling. We also suggest that the rearrangement of p53 and Th17 expression obtained in our preliminary experiments is a result of actin translocation from the cytoplasm into the nucleus and is probably mediated by micro-RNAs/long non-coding RNAs and possibly interfered when anesthetics are applied.We further suggest that the delay in differentiation is a result of Vangl2 signalings effect on the expression of Cx43.We also conclude that results obtained in these experiments can be used for designing vaccines based on RNA that hopefully can prevent neurodegenerative diseases as well as other diseases such as cancer.

P-30. THE NOVEL "UPPSALA" APP MUTATION CAUSES VERY EARLY-ONSET FAMILIAL ALZHEIMER'S DISEASE BY INCREASING Aβ FIBRIL FORMATION

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Early onset forms of AD can be caused by mutations in either of three genes - amyloid precursor protein (APP), presenilin 1 (PSEN1) or presenilin 2 (PSEN2). We have previously identified the Swedish and Arctic APP mutations that are pathogenic by increasing wild type Aβ (Aβwt) and by increasing toxic AβArc protofibrils, respectively.

Recently we have discovered the Uppsala mutation, a deletion of six amino acids in APP (APPUpp), in a family with autosomal dominant early onset AD. The Aβ aggregation kinetics was investigated with thioflavin T assay as well as with electron microscopy and revealed that the mutated Uppsala peptide (AβUpp) accelerates the formation of fibrils as compared to Aβwt and AβArc, which mainly consist of the longer AβUpp form (AβUpp36). Moreover, in cell-based experiments we have studied the possible effect of APPUpp on α-secretase and found preliminary evidence that the mutation leads to altered cleavage by this enzyme, which indicates that the Uppsala mutation could be pathogenic also by modifying α-secretase cleavage.

Immunohistochemistry in human brain slides revealed that plaques are abundant in parietal, temporal and occipital cortices and laser dissection mass spectrometry showed that they are mainly composed of AβUpp36. We have also generated a mouse line that overexpresses APPUpp and preliminary observations suggest that also brains from these mice form plaques that mainly consist of AβUpp36

P-31. IS THERE A DIRECT CAUSAL EFFECT OF EDUCATION ON DEMENTIA? A SWEDISH NATURAL EXPERIMENT ON 1.3 MILLION INDIVIDUALS

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Education is inversely associated with dementia risk, but it is unclear if this relationship is causal. We aimed to study causality of this relationship by exploiting a Swedish compulsory schooling reform, which extended education by 1 year for 70% of the population as a natural experiment. The reform introduced substantial exogenous variation in education unrelated to pupils' characteristics. We followed 18 birth cohorts (n=1,341,842) for nearly 30 years for dementia diagnosis in the National Inpatient and Cause of Death Registers. Our analyses indicated very small or negligible causal effects of education on dementia risk (HR = 1.01; 95% CI = 0.98-1.04). The results were robust against multiple sensitivity checks. The reform primarily captures direct causal effect because it had limited effects on further adult socio-economic outcomes. In absence of such mediating effects, education cannot be uncritically considered as a modifiable risk factor for dementia.vention and Reversal Model

P-32. USING A WIDE ARRAY OF AMYLOID PROBES FOR TAU FIBRIL POLYMORPH HYPERSPECTRAL FINGERPRINTING

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Background

One of the major challenges in Alzheimer's research today is to faithfully generate in vitro fibrils that exhibit the same properties and morphology as fibrils derived from patient samples [1]. It has been shown that it is possible to control fibril structure by using templating seeds [2], suggesting it would be feasible to generate more pathologically relevant fibrils in vitro by amplifying seeds from patient samples. However, there are currently no method that enables quick and simple characterization of fibril morphotype that is feasible to use in biological ex vivo samples as well as in in vitro preparations.

Aim

The aim of this study is to develop a method for characterization of fibril morphology based on fluorescence characteristics of different fluorescent amyloid probes when bound to the fibrils. By utilizing differences in fluorescence intensity and wavelength shift between the different fibril structures, we aim to generate a unique spectral fingerprint for each morphotype.

Methods

To generate hyperspectral fingerprints, probes from different amyloid ligand classes (bensothiadiazoles[3,4], bis-styryls, oligothiophenes[5]) were incubated with different in vitro generated fibrils. Emission spectra were collected in a fluorescence plate reader. The resulting data was analyzed in R.

Results & Discussion

Employing a battery of ligands from different classes, we recorded specific differences in the fluorescence characteristics of probes between different fibril morphotypes. This demonstrates the potential of using hyperspectral fingerprints for fibril characterization and differentiation as a versatile tool to move between in vitro and ex vivo samples.

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P-33. EVALUATION OF BISPECIFIC ANTIBODY BINDS TO ALPHA-SYNUCLEIN

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The aim of the project is to develop in vitro and in vivo methods to evaluate novel Positron Emission Tomography (PET) radioligands for visualizing aggregated pathological alpha-synuclein (α-syn) as a PET diagnostic marker for Parkinson's diseases (PD). In the present project, we specifically aim to develop autoradiography protocol to detect α-syn pathology with radiolabeled antibodies and to investigate bispecific antibody binding characteristics towards α-syn. And further to compare these radioactivity based methods with immunohistochemistry.

Our group has developed bispecific antibodies for PET imaging soluble aggregates of amyloid-beta (Ab) through transferrin receptor (TfR) -mediated transcytosis. We use TfR as a shuttle to bring antibodies into the blood-brain barrier (BBB) and reach a comparable concentration as small, lipophilic radioligands. We are now focusing on the mAbs targeting α-syn aggregates using the same shuttle, some of the radioligands candidates have been generated. We tested three candidates using in vitro autoradiography. Results showed specific bindings for three candidates. Besides, bispecific antibodies have a higher binding compared to their unmodified format. We could also found binding decrease when co-incubate with TfR binding. In the future, we will continue optimizing the autoradiography protocol, and investigating the binding affinity of candidates to different α-syn species.

P-34. COSTS OF STROKE REHABILITATION IN PATIENTS WITH DEMENTIA: A SWEDISH REGISTER-BASED STUDY

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Stroke and dementia are frequent co-morbidities in Sweden. Stroke rehabilitation constitutes a large proportion of direct health care costs for stroke. Dementia is possibly associated with different rehabilitation costs after stroke. However, this issue is still understudied.

Our study aims to explore cost of stroke rehabilitation for Swedish dementia vs. non-dementia patients and we conclude that dementia was significantly associated with lower stroke rehabilitation costs, despite worse health status and activities of daily living.

P-35. THE EFFECT OF RETROMER DYSFUNCTION ON THE CLEARANCE AND TRANSFER OF INTRA - AND EXTRA-CELLULAR BETA-AMYLOID IN NEURONS

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Objectives

The vacuolar sorting protein 35 (VPS35) is a central component of the retromer system, which is responsible for intracellular cargo sorting and recycling. A reduction in this protein was detected in the hippocampus of late-onset Alzheimer's disease (AD) patients. The objective of this study was to determine the effect of retromer dysfunction on the accumulation, transfer and clearance of oligomeric Aβ in neurons.

Methods

We established a stable cell line with retromer dysfunction by selectively inhibiting VPS35 gene expression using siRNA and shRNA in differentiated neuronal SH-SY5Y cells. Since VPS35 knockout is lethal, we also generated a cell line using CRISPR, which expresses truncated VPS35 and lacks the functional domain of the protein. We then investigated the effect of VPS35 reduction on the accumulation, transfer and clearance of oligomeric Aβ in a 3D co-culture system that allows the quantification of cell-to-cell protein transfer between donor and recipient cells.

Results

We show that retromer dysfunction increases oligomeric Aβ accumulation in neuronal cells and decreases clearance regardless if the oligomeric Aβ originates from extracellular milieu or direct neuronal transfer. The oligomeric Aβ also colocalises with VPS35 and early endosome markers suggesting the involvement of the endosomal pathway in the uptake of oligomeric AβOur model of retromer dysfunction also exhibits mitochondrial dysfunction and fragmentation, similar to that induced by a mutation in VPS35.

Conclusions

These findings provide evidence that retromer dysfunction decreases the ability of neurons to transport and clear neurotoxic Aβ oligomers resulting in their accumulation. Together with current evidence, our data points to the retromer system as an attractive therapeutic target to enhance the proper recycling and clearance of toxic oligomers to slow disease progression.

P-36. AMYLOIDOGENIC NANOPLAQUES IN BLOOD SERUM OF PATIENTS WITH ALZHEIMER'S DISEASE REVEALED BY TIME-RESOLVED THIOFLAVIN T FLUORESCENCE INTENSITY FLUCTUATION ANALYSIS

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Background
Biomarkers are central to current research on molecular mechanisms underlying Alzheimer's disease (AD). Their further development is of paramount importance for understanding pathophysiological processes that eventually lead to disease development. Biomarkers are also crucial for early disease detection, before clinical manifestation, and for development of new disease modifying therapies.

Objective
The overall aim of this work is to develop a minimally invasive method for fast, ultra-sensitive and cost-effective detection of structurally modified peptide/protein self-assemblies in the peripheral blood and other biological fluids. Specifically, we focus here on using this method to detect structured amyloidogenic oligomeric aggregates in the blood serum of apparently healthy individuals and patients in early AD stage, and measure their concentration and size.

Methods
Time-resolved detection of Thioflavin T (ThT) fluorescence intensity fluctuations in a sub-femtoliter observation volume element was used to identify in blood serum ThT-reactive structured amyloidogenic oligomeric aggregates, hereafter called nanoplaques, and measure with single-molecule sensitivity their concentration and size.

Results
The concentration and size of structured amyloidogenic nanoplaques are significantly higher in the blood serum of individuals diagnosed with AD than in control subjects.

Conclusion
A new method with the ultimate, single-molecule sensitivity was successfully developed [1]. The proposed approach neither relies on the use of immune-based probes, nor on the use of radiotracers, signal-amplification or protein separation techniques, and provides a minimally invasive test for fast and cost-effective early determination of structurally modified peptides/proteins in the peripheral blood, as shown here, but also in other biological fluids. Repeated sampling and analysis could inform the temporal development of pathology. Ultimately, this could lead to earlier diagnosis, earlier intervention and improved health outcomes for sufferers of AD.

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P-37. ALCOHOL ABUSE IMPAIRS NEUROGENESIS AND CAUSES NEURONAL LOSS IN HUMAN HIPPOCAMPUS – A MORPHOLOGICAL SUBSTRATE OF DEMENTIA?

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Objectives
Chronic alcohol abuse causes cognitive impairments associated with alcohol dementia. Hippocampal atrophy is especially pronounced. We hypothesize that alcohol abuse may have a detrimental effect on the neurogenic pool and may accelerate neuronal loss in the dentate gyrus.

Methods
Hippocampal samples were isolated from deceased donors with an on-going alcohol abuse during the four weeks prior to death and from controls with no alcohol overconsumption. A sample from the mid-portion of hippocampus was sectioned, immunostained for the neuronal nuclear marker NeuN, for Ki67, a marker for cell proliferation, Sox2, a stem/progenitor cell marker, and DCX, a marker for immature neurons, and counter stained with hematoxylin-eosin. Granule cell number and volume of granular cell layer in the dentate gyrus were estimated using stereology on donors with well-documented history of chronic alcohol abuse and controls with no alcohol overconsumption.

Results
Our data showed layer specific reduced numbers of all four markers in the dentate gyrus in subjects with an on-going alcohol abuse. This reduction of progenitor cells was most prominent in the subgranular zone. Moreover, loss of mature granular neurons and reduction of the granular cell layer volume were significant in comparison with controls.

Conclusion
Alcohol abusers show a pronounced reduction of stem/progenitor cells while loss of immature neurons is most likely a secondary effect. Loss of mature neurons in the alcoholic group could be explained either by an increase of cell death or by reduction of new cells added to the granular cell layer, or both. Accelerated neuronal loss in the hippocampus may represent the morphological substrate that contributes to alcohol-related cognitive impairment and onset of dementia.

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P-58. PATTERNS AND RATES OF COGNITIVE DECLINE DURING THE PRECLINICAL PHASE OF DEMENTIA: 12-YEAR FOLLOW-UP OF A POPULATION-BASED SAMPLE

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Background
Cognitive deficits can occur years or even decades before the clinical diagnosis of dementia, with a rate of decline that differs from that observed in normal aging. This study aimed to investigate differences in trajectories of cognitive decline in multiple cognitive domains in the preclinical dementia phase, and compare this to normal aging in a longitudinal, population-based setting.

Methods
Neuropsychological assessment was conducted for 1652 participants (age ≥60 years) from the Swedish National Study on Aging and Care–Kungsholmen (SNAC-K), who were free from dementia at baseline and at least one more follow-up assessment. 220 developed dementia and 1432 remained dementia free or died without dementia over the 12 years. Cognitive domains included episodic memory, semantic memory, verbal fluency, perceptual speed, and executive function. Age-, sex-, and education-adjusted linear mixed models and splines were used to compare rates of cognitive decline between people who developed dementia and those who remained cognitively intact across the follow-up period (reference group).

Results
Significantly faster decline was observed in all five cognitive domains (episodic memory, semantic memory, verbal fluency, perceptual speed, and executive functioning) in those in a preclinical phase of dementia compared to the reference group. In spline-models, participants in a preclinical dementia phase showed disproportionally accelerated decline as they neared the time of diagnosis in all cognitive domains except episodic memory; the rate of decline for episodic memory remained linear throughout the preclinical phase.

Conclusions
People in a preclinical dementia phase show accelerated rates of cognitive decline compared to normal aging. In addition, all cognitive domains but episodic memory show accelerated decline while approaching the dementia diagnosis. This pattern indicates an increase in pathological burden and/or a breakdown of compensatory mechanisms in the final years before a diagnosis of dementia.

P-39. BRAIN PHARMACOKINETICS OF BISPECIFIC ANTIBODY-BASED PET LIGANDS IS SIZE-DEPENDENT

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Objectives
Antibodies targeting amyloid-beta (Aβ) could be used in positron emission tomography (PET) to study Alzheimer's disease pathology. Challenges are: low brain penetrance and slow blood elimination. Bispecific antibodies utilising the mouse transferrin receptor (mTfR) are transported into the brain in high amounts. However, knowledge of their brain clearance time is lacking. The aim was to study brain pharmacokinetics of bispecific antibody-based ligands and to label them with 18F for PET.

Methods
The bispecific antibody ligands mAb3D6-scFv8D3 (210 kDA) and di-scFv3D6-8D3 (58 kDa) were expressed in Expi293-cells and in vitro affinity to Aβ protofibrils and mTfR were confirmed by ELISA. The ligands were 125I-labelled and each of them administered to WT and APP transgenic mice. Brains were isolated 2 h-24 h after injection and radioactivity was measured. Autoradiography was performed on sagittal brain sections. PET was performed with 18F labelled ligands.

Results
The brain half-life of [125I]mAb3D6-scFv8D3 and [125I]di-scFv3D6-8D3 were 8 h and 3 h respectively. Autoradiography and PET signals were 20% higher in the frontal cortex of transgenic animals compared to WT, 10-11 h after injection, indicating Aβ-specific signal. However, unspecific signal from blood and non-Aβ bound ligand in the brain was high.

Conclusions
Aβ pathology could be visualised using 125I or 18F labelled brain-penetrating antibodies. However, images suffered from unspecific signal. Smaller non-IgG-like antibody-based constructs, such as di-scFv3D6-8D3, seem more promising as 18F labelled PET radioligands. They have a shorter half-life in blood and brain compared with IgG-like bispecific antibodies, and should be developed further.

P-40. AUTOPHAGY-DEFICIENCY IN NEURONS INDUCES INTRACELLULAR AGGREGATION OF ALZHEIMER-ASSOCIATED AMYLOID BETA INTO FIBRILS AND NEURODEGENERATION IN ALZHEIMER MOUSE MODELS

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Alzheimer's disease (AD) is the major form of dementia and is characterized by memory loss and cognitive decline. AD is caused by the pronounced amyloid beta (Amyloidbeta and tau pathologies in the brain which induce the severe neurodegeneration and onset of clinical symptoms. Autophagy is impaired in AD as shown by accumulation of autophagosomes in dystrophic neurites. However, it is not known whether this is caused by an induced autophagy or an impaired end stage lysosomal clearance nor if the alterations change with disease progression. Nestin-mediated genetic deletion of autophagy in neurons in the mouse brain leads to neurodegeneration showing an absolute requirement for functional autophagy for neuronal maintenance and survival. To elucidate the mechanism of autophagy-mediated neurodegeneration and its interplay with amyloidbeta, we generated conditional knockout mice by deleting autophagy-related gene 7 (Atg7) in excitatory neurons. Genetic deletion of Atg7 induced neurodegeneration through caspase 3 activation. To investigate the effect of a concomitant deletion of autophagy and a robust amyloidbeta pathology, we crossed Atg7 cKO mice with novel amyloid precursor protein (APP) knock-in AD mice that are free of APP overexpression employed in previous APP transgenic mice. Interestingly, deletion of autophagy leads to intracellular accumulation of amyloidbeta which assemble into fibril structures, as shown by immunoelectron microscopy, previously only observed in extracellular Amyloidbeta deposits. The intracellular Amyloidbeta exacerbates the neurodegeneration induced by autophagy-deficiency. We show this by histological means as well as MRI in living mice. The intracellular amyloidbeta accumulation is paralleled by significantly decreased extracellular amyloidbeta depositions due to decreased amyloidbeta secretion which is mediated by autophagy. Analysis of exosome content in the brains of the mice revealed decreased levels of LC3II-positive exosomes which may explain the reduced amyloidbeta secretion upon deletion of autophagy. Furthermore, deletion of Atg7 leads to impaired memory in the autophagy deficient APP mice. This shows that autophagy plays a key role in amyloidbeta metabolism and that lack of functional autophagy is detrimental for the memory

P-41. THE LINK BETWEEN SENESCENT ASTROCYTES TO THE PROGRESSION OF ALZHEIMER'S DISEASE

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Cellular senescence (CS) is a state in which cells cease to divide and undergo many morphological and functional changes. Among the changes is the senescence associated secretory phenotype (SASP) which includes pro-inflammatory cytokines. The role of cellular senescence (CS) has been shown in diseases such as cancer. Astrocytes are the most abundant cells in the brain, they are responsible for the maintenance and homeostasis of the brain, and many studies have shown their fundamental role in cognition. Alterations in astrocyte function may impair the brain function and its abilities. With age and in Alzheimer's disease (AD), senescent astrocytes accumulate and express a senescence-associated secretory phenotype. Here we aim to assess whether the activity of astrocytes was changed due to continuous exposure to beta amyloid leading to impairment in their activity. We found high senescence marker in astrocytes next to Aεplaques in age dependent manner 5xFAD mice. Furthermore, we found that incubation with Aε1-42 with adult astrocytes induces astrocyte senescence, suggesting that the increasing load of Aεin AD might contribute to the induction of astrocyte senescence. Furthermore, astrocytes isolated from old 5xFAD mice secrete higher levels of IL-6 and fail to support neurons in NF-ɮb dependent manner. Our results suggest that chronic exposure Aεcan contribute to astrocyte senescence and may play a crucial role in AD pathology

P-42. INCREASED BRAIN UPTAKE OF A BISPECIFIC ANTIBODY TARGETING ALPHA-SYNUCLEIN

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Antibody-based strategies are emerging for targeting oligomeric, toxic forms of alpha-synuclein, believed to be central in pathological processes underlying Parkinson's disease (PD). However, antibodies are large molecules and require engineering for increased brain penetration. Previously, we have utilized the transferrin receptor (TfR), a carrier-mediated transporter expressed at the blood-brain barrier (BBB) to boost brain delivery of an amyloid-beta targeting antibody. Here, we sought to employ the same format for targeting oligomeric alpha-synuclein.

Methods
We have recombinantly expressed a bispecific antibody with affinity for both oligomeric alpha-synuclein and TfR. This format is accomplished by fusing the antibody with two single-chain variable fragments (ScFv) of 8D3, an anti-TfR antibody, to the C-terminal end of the light chains with short linkers. The linkers sterically hinder bivalent binding of ScFv8D3 to the TfR dimer, believed to be disadvantageous for the essential dissociation from TfR on the abluminal side of the BBB. Antibody functionality in vitro was assessed with ELISA and autoradiography on brain sections from Thy-1 aSyn ("Line 61") mice overexpressing wildtype human alpha-synuclein as well as wildtype mice, and in vivo by measurements of 125I-labeled antibody in wildtype mice.

Results
Binding properties of the bispecific antibody showed specific binding to TfR, whereas binding to oligomeric alpha-synuclein remained unaltered in comparison with unmodified antibody as measured by ELISA. In vitro autoradiography on mouse brain sections of Line 61 mice displayed specific binding to alpha-synuclein as compared with wildtype sections. Intravenous administration of bispecific antibody in wildtype mice showed more than 50 times higher brain uptake than unmodified antibody, with concentrations of approximately 2% of injected dose per gram brain tissue 2 hours post-administration.

Conclusions
Fast and high brain distribution of the bispecific alpha-synuclein antibody is comparable to small brain penetrant molecular drugs, presenting promising possibilities for both diagnostics and therapeutics of PD.

P-43. THE DRUG COPAXONE, USED TO TREAT MULTIPLE SCLEROSIS (MS), SIGNIFICANTLY REDUCES THE TOXICITY OF AMYLOID-BETA PEPTIDE AGGREGATES

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The drug copaxone has for several decades been successfully used to treat relapsing forms of multiple sclerosis (MS), but its mechanism of action remains unknown. Copaxone consists of random sequences of variable length of the four amino acids alanine, glutamate, lysine, and tyrosine. These random peptide sequences can self-assemble into fibrils that are similar to those formed by amyloid proteins such as amyloid-beta (Aβ, involved in Alzheimer's disease) and β-synuclein (involved in Parkinson's disease). Here, we have studied the effects of copaxone on Aβ aggregation and toxicity. Our ThT fluorescence assays and AFM images show that copaxone has a profound impact on the Aβ aggregation process, possibly due to co-aggregation effects. Our cell toxicity studies show that copaxone significantly reduces the toxicity of Aβ oligomeric aggregates. Taken together, these results suggest that the already tested and approved drug copaxone might be used to treat also Alzheimer's disease.

P-44. DEVELOPMENT OF A BISPECIFIC BRAIN PENETRATING TREM2 ANTIBODY

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Objectives
The microglial protein Trem2 has recently been associated with Alzheimer's disease and Aβ pathology. It is therefore a potential target both diagnostic development and therapy. We are developing a bispecific antibody, targeting Trem2 and the transferrin receptor (TfR) to enable receptor mediated transcytosis across the blood-brain barrier.

Methods
To investigate the relation of Trem2 and Aβ pathology, brain extracts from APP transgenic (tg-ArcSwe) and wildtype (wt) mice of different ages were analyzed with ELISAs specific for soluble Trem2 and Aβ aggregates. An anti-Trem2 antibody was then chemically conjugated to a single chain fragment (scFv) of the TfR antibody 8D3 to generate a bispecific, brain penetrating antibody with the ability to specifically target Trem2 inside the brain. The bispecific antibody was radiolabeled with iodine-125 to for ex vivo studies of brain uptake.

Results
Brain concentrations of soluble Trem2 increased with age in tg-ArcSwe mice, while it remained low in wt mice of all ages. Trem2 thus correlated well with Aβ pathology, which increased with age and genotype in a similar manner. The radiolabeled bispecific antibody demonstrated a significantly increased brain uptake in wt mice, compared with non-modified antibodies.

Conclusion
This study demonstrates that Trem2 is significantly elevated in tg-ArcSwe mice, closely following the underlying Aβ pathology, in line with previous reports. Trem2 is therefore a potential target for both diagnosis and therapy of AD and the bispecific brain penetrating Trem2 antibody developed here may be an important tool in this development.

P-45. IN VITRO AND IN VIVO CHARACTERIZATION OF SIGNAL PEPTIDE PEPTIDASE LIKE 2 b (SPPL2b): A NOVEL INTRAMEMBRANE ENZYME INVOLVED IN ALZHEIMER´S DISEASE

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Introduction
Alzheimer´s disease (AD) is an age-related neurodegenerative disease characterized by the accumulation of amyloid β-peptide (Aβ) in plaques and neurofibrillary tangles. Identifying new mechanisms and proteins involved in AD pathogenesis is a crucial step to find new therapeutic targets. The intramembrane enzyme Signal Peptide Peptidase Like 2b (SPPL2b) is a potential target since it is involved in the regulation of proteolysis of two AD-related proteins: TNF-alpha and BRI2 involved in the inflammatory response and Aβ production respectively, BRI2 interacts with APP and blocks access of secretases to the cleavage sites. SPPL2b is highly expressed in the brain and in particular in the hippocampus. Here we have investigated for the first time the expression levels and the pathogenic role of SPPL2b in AD by using both in vitro and in vivo assays.

Methods
The pathophysiological role of SPPL2b in AD was evaluated in vitro by using human neuroblastoma cell line SH-SY5Y stably expressing APP and thus generating high levels of Aβ, brain slices from WT mice and a new APP knock-in AD mouse model (AppNL-G-F). The levels of SPPL2b and its related substrates and Aβ production were evaluated by Western blot and ELISA.

Results
APP overexpression in SH-SY5Y cells increased the levels of SPPL2b. Interestingly, APP overexpression reduced BRI2 levels due to increased processing of BRI2. A reduction tendency in Aβ40 production was observed in WT and APP_SH-SY5Y cells when treated with the selective SPPLs enzyme inhibitor (Z-LL)2 –ketone. In cortex brain sections maintained ex vivo, Aβ42 exposure induced a strong up-regulation of SPPL2b. High levels of SPPL2b were also observed in the early stage of the AD associated Aβ pathology of 2-3 months old AppNL-G-F mice in the cortex. However, at 10-14 months of age SPPL2b protein expression was significantly lowered when compared to control mice.

Conclusion
These results strongly support the involvement of SPPL2b in AD pathology, where an up-regulation in the early stages can play a kay role in the onset of the diseases by promoting both the inflammatory and amyloidogenic pathway. Most important, Aβ42 seems directly involved in SPPL2b expression.

P-46. NANOSPECTROSCOPY OF AMYLOID-β: CHALLENGES AND REWARDS

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Infrared Spectroscopy is a powerful non-invasive tool to study secondary structures in amyloid and other misfolded proteins (Barth 2007). Infrared scattering-type scanning near-field optical microscopy (IR s-SNOM) is a recent advancement where it is combined with atomic force microscopy (AFM) operating in tapping mode. The spatial resolution (<20 nm) is determined by the sharp metallic AFM tip, and it is far superior compared to ordinary infrared microscopy. In our research, we study the oligomeric and fibrillar structures of Aβ40 and 42 using this cutting-edge nanoscale infrared technique.

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P-47. EFFECTS OF Cu(II) IONS ON THE AMYLIN/IAPP AGGREGATION PROCESS

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Aggregation of peptides and proteins into amyloid material is a hallmark of a number of diseases, such as Alzheimer's disease (amyloid-beta (Aβ) peptides), Parkinson's disease (α-synuclein), and diabetes (amylin/IAPP). Cross-aggregation between these peptides and proteins might explain why many of these diseases often occur together, such as diabetes and Alzheimer's. It is therefore important to investigate common factors that may affect the aggregation processes of the peptides and proteins involved in these diseases. One important common factor appears to be metal ions, appear to affect the aggregation of many proteins. Metal dyshomeostasis is furthermore a common condition in many amyloid diseases, together with oxidative stress, which often is induced by redox-active metal ions such as Cu(II) and Fe(II). Here, we have used various biophysical methods including ThT fluorescence assays, tyrosine fluorescence quenching, and atomic force microscopy (AFM) imaging to characterize in vitro the binding of copper ions to the amylin/IAPP peptide, and the effects of such copper binding on the amylin/IAPP aggregation process. The binding effects were investigated in different environmental conditions, such as neutral and acidic pH, oxidative and reducing environments, and aqueous and membrane-mimetic environments. Our results show that copper ions bind to the amylin/IAPP peptide with micromolar binding affinity, and modulate the amylin/IAPP aggregation already at stoichiometric concentrations. These binding effects are less pronounced at low pH and in a reducing or membrane-like environment.

P-48. 2ND GENERATION NGF RELEASING CELLS RESISTS DYSREGULATION FOLLOWING EXPOSURE TO Aβ40/42 AND ASTROGLIAL ACTIVATION: AN UPDATE ON ECB-NGF THERAPY FOR ALZHEIMER'S DISEASE

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Objectives
Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterised by the loss of cholinergic neurons in the basal forebrain. Since, nerve growth factor (NGF) is required for the maintenance and survival of cholinergic neurons, its targeted delivery via encapsulated cell biodelivery device (ECB) was envisaged as potential therapeutic strategy. In our first-in-human studies, we observed some inter-capsule differences in NGF release among ECB-NGF devices, when implanted in human AD patients. To address this issue, clarity is required whether amyloid-beta (Aβ) peptides and inflammatory molecules can affect the NGF-delivering (NGC-0211) cells which are present inside the implanted ECB's.

Methods
We evaluated the direct and astrocyte-mediated effect of Aβ40 and Aβ42 on the NGC-0211 cells in-vitro. To study the indirect effect of Aβ peptides, human primary astrocytes were first exposed to different concentrations of Aβ peptides and the conditioned medium was further transferred on NGF0211 cells to study effects on cell viability, biochemical parameters and NGF release.

Results
We report that both Aβ-peptides induce marginal effect on cell survival and that conditioned media obtained from Aβ-exposed astrocytes partially modulate biochemical parameters. In both cases, the mitochondrial connectivity of the NGC-0211 cells was impaired during early time points. Lastly, Aβ exposure (direct and indirect) did not hamper the NGF production from the NGC-0211 cells.

Conclusions
Our data suggest that the survival of the NGC-0211 cells was only marginally impaired by the toxicity of Aβ peptides, while the NGF release was unaffected following exposure with Aβ peptides directly or via astrocyte activation indirectly.

P-49. ALUMINUM AND ALZHEIMER'S DISEASE: Al(III) IONS DO NOT BIND SPECIFICALLY TO THE AMYLOID-β (Aβ) PEPTIDE

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Background
Some studies have suggested a role for aluminum (Al) in the pathogenesis of Alzheimer disease (AD), but few studies have been carried out to investigate the possible binding of Al(III) ions to the amyloid-β (Aβ) peptide, which is known to bind other metal ions.

Materials and methods
Nuclear magnetic resonance (NMR) spectroscopy was used to characterize the binding of Al(III) ions to Aβ(1-40) peptides. Circular dichroism (CD) and thioflavin T (ThT) fluorescence were used to investigate the aggregation kinetics of Aβ(1-40) in the presence of varying concentrations of Al(III), and atomic force microscopy (AFM) imaging was used to evaluate the morphology of the peptide aggregates.

Results and conclusions
Al(III) ions do not bind to specific Aβ(1-40) residues even at an Al(III):Aβ(1-40) ratio of 10:1, and do not significantly influence the Aβ aggregation kinetics or alter the Aβ fibril morphology even at high concentrations.

P-50. CONFORMATIONAL VARIATION OF TAU FILAMENTOUS INCLUSIONS AND THE DEVELOPMENT OF MULTIMODAL LIGANDS – ONE SIZE DOES NOT FIT ALL

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Introduction
Abnormal assembly of tau filamentous inclusions can be seen in several neurodegenerative diseases. The inclusions are composed of either the 3R or 4R tau isoform, or a combination of both, and it was recently shown that tau filaments adopt disease-specific conformations. We are developing tau ligands, and the purpose of this study was to investigate if they can be used to separate distinct conformers of tau.

Methods
Human brain sections from neuropathologically characterized cases of Alzheimer´s disease (AD), chronic traumatic encephalopathy (CTE), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), Pick's disease (PiD) or MAPT deltaK280 were stained with ligand bTVBT3, q-FTAA-CN or HS-276. Double staining with tau AT8 antibody was performed to confirm specificity.

Results
Ligand bTVBT3 showed binding to tau in AD, whereas q-FTAA-CN and HS-276 were abeta selective. In CTE, bTVBT3 only labelled a small subset of AT8-positive tau aggregates, while q-FTAA-CN demonstrated almost complete co-labelling with antibody. q-FTAA-CN also stained all types of tau pathologies in PSP, CBD, PiD and MAPT deltaK280 brain tissues, whereas bTVBT3 mainly labelled neuropil threads. HS-276 only stained non-AD tau.

Discussion
The variation in binding affinity indicates that the ligands can be used to separate disease-specific conformers of tau. Ligand staining demonstrated a conformational difference between AD and CTE tau inclusions, which was recently also shown when comparing tau filament structures using cryo-EM. Our results suggest that inclusion conformation is not dependent on tau isoform but on tau pathology type.

P-51. SPECT IMAGING AND BRAIN RETENTION OF BRAIN PENETRATING BISPECIFIC AND UNMODIFIED ANTIBODIES IN AN AMYLOID BETA MOUSE MODEL

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Objectives
We have evaluated long-term brain retention and distribution of a brain penetrating, bispecific antibody targeting Abeta protofibrils and the TfR (used as a brain shuttle), RmAb158-scFv8D3, in comparison with unmodified RmAb158 in tg-ArcSwe and WT mice.

Methods
RmAb158-scFv8D3 and RmAb158 were labeled with iodine-125 (125I) and administered i.v. to old tg-ArcSwe and WT mice. Blood pharmacokinetics were evaluated over a period of 27 days and SPECT scans were performed at 6, 14 and 27 days. Brain was isolated following SPECT and radioactivity was measured ex vivo. Autoradiography and micro autoradiography, combined with Congo red staining, were performed on brain sections to investigate antibody intrabrain distribution and Abeta plaque interaction.

Results
[125I]RmAb158-ScFv8D3 showed a faster blood clearance compared to [125I] RmAb158. However, [125I]RmAb158-scFv8D3 displayed higher brain retention at all time points (Figure). SPECT and autoradiography showed a more uniform distribution, coinciding with Abeta pathology, of [125I]RmAb158-scFv8D3 compared with [125I]RmAb158. Micro autoradiography indicated greater vascular escape and plaque interaction for the bispecific antibody.

Conclusion
RmAb158-scFv8D3 showed higher brain concentrations than unmodified RmAb158 at all time points, demonstrating the feasibility of TfR mediated transcytosis. The global distribution pattern in the brain parenchyma was fundamentally different between the two types of antibodies; RmAb158-scFv8D3 was detected throughout the brain in line with the abundant brain Abeta pathology while RmAb158 appeared in a more scattered pattern, at later time points concentrated to ventricular areas.

P-52. ASSOCIATION OF EEG TOPOGRAPHICAL MARKERS WITH FDG-PET BRAIN GLUCOSE METABOLISM AND CSF NEUROGRANIN IN MCI AND AD PATIENTS

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Objectives
Synaptic dysfunction is the best correlate of cognitive impairment that precedes neuronal cell death in patients along Alzheimer's disease (AD) continuum. Fluorodeoxyglucose positron-emission tomography (FDG-PET) and electroencephalography (EEG) measure indirect metabolic and direct real-time synaptic functioning, respectively, implicating their potential to serve as valuable diagnostic and/or prognostic markers of AD. The present study investigated correlation of EEG topographical measures with the pattern of brain glucose hypometabolism and cerebrospinal fluid (CSF) synaptic marker neurogranin in mild cognitive impairment (MCI) and AD patients.

Methods
Patients diagnosed with MCI (n=49) and AD (n=24) were clinically assessed with FDG-PET imaging, resting state EEG and CSF neurogranin analysis. EEG-based neuroimaging involved low-resolution electromagnetic tomography (LORETA) which estimates cortical sources of electrical activity in a 3D human head model. Both EEG LORETA connectivity and glucose standardized uptake value ratios were calculated for the five main brain lobes.

Results
The preliminary results of our ongoing analysis show that the decreased brain functional connectivity, assessed by EEG LORETA, correlates with increased CSF neurogranin levels and decreased brain glucose metabolism in temperoparietal lobes in MCI and AD patients.

Conclusions
EEG topographical markers correlate with surrogate synaptic markers and therefore contribute to early detection of synaptic dysfunction in patients along AD continuum. EEG is a widely available and noninvasive diagnostic method that might have broad implication in AD drug trials and clinical practice.

P-53. WHAT IF ROOT CAUSE OF ALZHEIMER'S DISEASE-DEMENTIA AND ROOT CAUSE OF VIOLENCE SHARE AN INTEGRATED FETAL BIOGRAPHY AND LEGAL PATHOLOGY?

Dr. Windell Matthews

Introduction
Cases of Alzheimer's Disease – dementia (Alzheimer's) and acts of violence are rising within regions around the globe. With husbands and wives both being diagnosed with Alzheimer's, the next two decades will witness a massive dementia care crisis.

Women dominate Alzheimer's statistics after age 60; that domination need not be the case.

Men, dominating Alzheimer's global statistics within pre-geriatric ages 30-59, hold professional and blue collar jobs and give the appearance of leading lives without any hint of having Alzheimer's. This cohort has the highest rate of domestic violence and femicide.

Diagnosed with Children's Alzheimer's, children suffer memory loss, dementia, and forget how to walk and talk, suggesting Alzheimer's is a pediatric issue.

This researcher found clusters of Alzheimer's within clusters of violence.

Purpose
The purpose is three-fold: (1) to determine whether violence and Alzheimer's share root cause; (2) to identify the source of commonality; and (3) to test whether it is possible to reverse early stage Alzheimer's, using The Windell Alzheimer's Pre Education is inversely associated with dementia risk, but it is unclear if this relationship is causal.

P-54. CHARACTERIZATION OF Ni(II) ION BINDING TO THE AMYLOID-BETA(1-40) PEPTIDE: RESIDUE-SPECIFIC INTERACTIONS RETARD Aβ AGGREGATION

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Brain deposits of insoluble amyloid plaques consisting mainly of aggregated amyloid-β(Aβ) peptides are a hallmark of Alzheimer's disease (AD), the most common cause of dementia worldwide. A binding site for metal ions is present in the N-terminal Aβ segment, and the AD brain plaques are known to contain elevated levels of transition metals such as Cu, Fe, and Zn. Even though AD patients display altered metal homeostasis, the details of the metal chemistry involved in AD disease pathology remain unresolved. Previous studies suggest that Ni(II) ions may interact with Aβ peptides, but the effects of such interactions remain to be clarified. According to the Irving-Williams series, Ni(II) ions are expected to display weaker protein binding than Cu(II) ions, but stronger than Mn(II), Fe(II), and Co(II) ions. So far, the Aβ/Ni(II) binding affinity has not been quantified. Here, we use nuclear magnetic resonance (NMR) and fluorescence spectroscopy together with atomic force microscopy (AFM) imaging to show that Ni(II) ions display specific binding to the N-terminal Aβ segment, and that such binding retards the Aβ aggregation process and directs it towards non-fibrillar aggregates. The His6, His13, and His14 residues are implicated as binding ligands, and the Ni(II)/Aβ binding affinity is in the range 10-250 μM.implication in AD drug trials and clinical practice.

P-55. DIFFERENTIAL ASSOCIATIONS OF BLOOD PRESSURE IN MIDLIFE AND OLD AGE WITH RISK OF INCIDENT DEMENTIA: A POPULATION-BASED STUDY

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Objective
To examine the associations of various blood pressure components in midlife and old age as well as their trajectories from mid- to late-life with incident dementia in one cohort.

Methods
This population-based cohort study included 1449 participants in the Finnish Cardiovascular Risk Factors, Aging and Dementia study, who were examined in 1972-1987 in midlife (mean age 50.4 years) and re-examined in 1998 (mean age 70.2 years), and the 742 survivors were re-examined in 2005-2008. Arterial blood pressure was measured at each examination. Dementia was diagnosed following the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria. Data were analyzed using multiple Cox proportional-hazards models.

Results
During the follow-up examinations, dementia was diagnosed in 61 persons in 1998 and 47 persons in 2005-2008. Midlife high systolic pressure and high pulse pressure were associated with multi-adjusted hazard ratios of 2.01 (95% confidence interval 1.16-3.49) and 2.49 (1.40-4.44), respectively, for dementia. None of the 4 blood pressure components in late-life was significantly associated with the risk of dementia. A decline of ≥15 mmHg in systolic pressure and mean arterial pressure from mid- to late-life was associated with multi-adjusted hazard ratios of 3.59 (1.76-7.32) and 2.32 (1.07-5.06), respectively, for dementia.

Conclusions
High systolic pressure and high pulse pressure in midlife but not in old age are associated with an increased risk of dementia. A substantial decline in systolic pressure and mean arterial pressure from mid- to late-life anticipates a substantial risk of dementia.

P-56. JOINT IMPACT OF COMMON RISK FACTORS ON INCIDENT DEMENTIA: A COHORT STUDY OF THE SWEDISH TWIN REGISTRY

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Background
The Lancet Commission has proposed a life-course model for dementia based on nine modifiable risk factors (low education, hearing loss, hypertension, obesity, smoking, depression, physical inactivity, diabetes, social isolation). However, the joint impact of these factors on incident dementia is still uncertain; hence, we aimed to examine this impact.

Methods
We conducted a cohort study of 9,017 cognitively-intact individuals aged ≥65 years, in the Swedish Twin Registry. The main exposure was the number of reported risk factors ranging from zero to nine. Data on dementia diagnoses were retrieved from Swedish national health registers. After estimating the multivariate-adjusted hazard ratios of incident dementia, the population attributable fraction (PAF) was calculated. We also conducted two additional analyses; including APOE ϵ4 status in a genotyped subsample (n=2,810) and discordant twin pair analysis (n=651 discordant pairs).

Results
Mean (SD) age at baseline was 72.1 (5.7) years. The number of incident dementia cases was 1,950 (21.6%). A dose–response relationship between the number of risk factors and incident dementia was observed; hazard ratio (95% confidence interval) per one unit increment in number of risk factors was 1.07 (1.03 to 1.11). The PAF for the combination of the nine risk factors was 10.4%. The PAF of APOE ϵ4 status was twice (20.8%) that of the nine risk factors combined. Discordant twin pair analysis suggested that the observed association was not explained by familial effects (shared genetic or familial environmental factors).

Conclusions
Our results suggest that the joint impact of the nine risk factors has potential as modifiable factors on incident dementia.

P-12. RESOLVING MICROGLIAL INFLAMMATION IN ALZHEIMER'S DISEASE - POTENTIAL TREATMENT TARGET?

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Introduction
Alzheimer's disease (AD) is the leading cause of dementia with pathological accumulation ofβ-amyloid (Aβ) and hyperphosphorylation of tau proteins, together with damaging chronic inflammation as indicated by activated microglia. Maresin 1 (MaR1) is a specialised pro-resolving lipid mediator facilitating resolution of inflammation and restoring homeostasis. Our study aims to explore the pro-resolving role of MaR1 in AD.

Methods
Differentiated THP-1 cells treated with Aβ42 was used as an AD model. Effects of MaR1 on Aβ42-induced pro-inflammatory cytokine secretion, cytotoxicity, intracellular kinase activation and pro-inflammatory surface biomarker elevation were analyzed by ELISA, LDH assay, Western Blot and flow cytometry, respectively. Phagocytosis of fluorphore-conjugated Aβ42 was measured by flow cytometry.

Results
Aβ42 induced an increase in the secretion of pro-inflammatory cytokines of IL-1β, TNF-β and IL-6, and this effect was significantly decreased upon co-incubation with MaR1. The anti-inflammatory cytokines IL-4 and IL-10 were not detectable in cell supernatant. Aβ42 treatment resulted in cell death, which was protected by co-incubation with MaR1. MaR1 reduced Aβ-induced pro-inflammatory surface biomarker CD40 elevation, while CD86 and anti-inflammatory surface biomarkers CD163 and CD200R were not affected. MaR1 significantly increased Aβ42 uptake by THP-1 cells. No effect of MaR1 on Aβ42-induced pro-inflammatory kinases p38 MAPK, p44/42 MAPK and SAPK/JNK activation was observed.

Conclusion
MaR1 plays a pro-resolving role in the context of AD by attenuating Aβ42-induced pro-inflammatory reactions and promoting Aβ42 clearance. MaR1 could be a potential therapeutic target for AD.

P-38. GALANTAMINE VERSUS RISPERIDONE TREATMENT OF NEUROPSYCHIATRIC SYMPTOMS IN PATIENTS WITH PROBABLE DEMENTIA: AN OPEN RANDOMIZED TRIAL

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Objective
To examine the effects of galantamine and risperidone on neuropsychiatric symptoms in dementia (NPSD) and global function.

Methods
Using a randomized, controlled and open-blind, one-center trial at an in- and outpatient clinic at a university hospital, we studied 100 adults with probable dementia and NPSD. Participants received galantamine (N ¼ 50, target dose 24 mg) or risperidone (N ¼ 50, target dose 1.5 mg) for 12 weeks. The primary outcome was effects on NPSD assessed by the Neuropsychiatric Inventory (NPI). Secondary measures included the Mini-Mental State Examination (MMSE), Clinical Dementia Rating, Clinical Global Impression, and Simpson Angus scales. All tests were performed before and after treatment.

Results
Outcome measures were analyzed using analysis of covariance. Ninety-one patients (67% women, mean age 79 7.5 years) with initial NPI score of 51.0 (25.8) and MMSE of 20.1 (4.6) completed the trial. Both galantamine and risperidone treatments resulted in improved NPSD symptoms and were equally effective in treating several NPI domains. However, risperidone showed a significant treatment advantage in the NPI domains irritation and agitation, $F(1, 97) = 5.2$, $p = 0.02$. Galantamine treatment also ameliorated cognitive functions where MMSE scores increased 2.8 points compared with baseline (95% confidence interval: 1.96e3.52). No treatment-related severe side effects occurred.

Conclusions
These results support that galantamine, with its benign safety profile, can be used as firstline treatment of NPSD symptoms, unless symptoms of irritation and agitation areprominent, where risperidone is more efficient.

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