demonstrated PZP immunoreactivity in the brain, predominantly localized to microglial cells. We observed an increased amount of PZP immunoreactive microglial cells in the AD brain compared to healthy controls and in addition, PZP immunoreactive cells were found in close association with senile plaques. PZP is best known as a serum protein that is upregulated during pregnancy and is capable of covalently binding and immobilizing various ligands mostly related to protease inhibition. The increased abundance and close proximity of PZP to AD plaques suggests involvement of protease inhibition during plaque formation. In this study we aim to investigate the pathophysiological role of PZP in AD by analyzing brain derived PZP and its interaction partners.

Methods: We will enrich senile plaques and PZP from human brain tissue using two techniques i.e. laser capture microdissection (LCM) and immunoprecipitation (IP). PZP will be quantified using nano liquid chromatography mass spectrometry (nLC-MS) based techniques and selective reaction monitoring (SRM). Ligand binding to PZP generates a shift in the molecular weight of such a complex. Peptides that are generated enzymatically by trypsin cleavage of a PZP-ligand complex generate information on how the ligand is bound covalently to PZP. In this manner we can identify PZP interaction partners and the biochemical process of binding.

Results: IP method optimization was performed using serum from pregnant women that contained high levels of PZP. We successfully enriched PZP from this material and identified a number of interaction partners using our approach. Experiments on human AD and control brain lysates and on brain samples isolated by LCM are ongoing. Conclusions: Our data indicate that PZP is a potential early player in AD that might contribute to plaque formation. Increased serum PZP might be indicative of an early inflammatory, microglia mediated process in the brain aimed at clearing aggregating factors. Failure of this mechanism, e.g. altered ligand binding or clearance, may contribute to plaque formation.

P2-057 INTERACTOME ANALYSES OF MATURE GAMMA-SECRETASE COMPLEXES REVEALS DISTINCT MOLECULAR ENVIRONMENTS OF PRESEN ILIN PARALOGS AND PREFERENTIAL BINDING OF SIGNAL PEPTIDE PEPTIDASE TO PS2

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Background: γ-Secretase plays a pivotal role in the production of neurotoxic amyloid β-peptides (Aβ) in Alzheimer’s disease (AD) and consists of a heterotrameric core complex that includes the aspartyl intramembrane protease presenilin (PS). The human genome codes for two presenilin paralogs. Methods: To elucidate whether PS mutations associated with early-onset AD affect the molecular environment of mature γ-secretase complexes and understand the causes for distinct phenotypes of PS paralog deficient mice, quantitative interactome comparisons were undertaken. Brains of mice engineered to express wild-type or mutant PS1, or HEK293 cells stably expressing PS paralogues with N-terminal tandem-affinity purification (TAP) tags served as biological source materials. Results: The analyses revealed novel interactions of the γ-secretase core complex with a molecular machinery that targets and fuses synaptic vesicles to celluar membranes and with the H + -transporting lysosomal ATPase macro-complex but uncovered no differences in the interactomes of wild-type and mutant PS1. The catenin/cadherin network was almost exclusively found associated with PS1. Another intramembrane protease, signal peptide peptidase (SPP), predominantly co-purified with PS2-containing γ-secretase complexes and was observed to influence Aβ production. Conclusions: The study confirmed the anticipation that a majority of presenilin candidate interactors associate with both PS1- and PS2-containing γ-secretase complexes but also revealed for the first time a small number of proteins that co-purified in a PS paralog-specific manner. It remains to be seen if the surprising association of PS2 and SPP serves the purpose to facilitate rapid re-moval of transmembrane stubs embedded in the lipid bilayer with opposite topologies.

P2-058 PYROGLUTAMYLATED BETA-AMYLOID IS ASSOCIATED WITH HYPERPHOSPHORYLATED TAU IN HUMAN POST-MORTEM BRAINS

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Background: Pyroglutamylation amyloid-β (pE-Aβ) is generated by N-terminal truncation of Aβ and subsequent cyclization of N-terminal glutamate by glutaminyl cyclase and has been suggested to play a major role in Alzheimer’s disease (AD) pathogenesis; recent data from transgenic animal and cell culture experiments suggest that Aβ oligomers containing pE-Aβ might initiate tau dependent cytotoxicity thereby establishing a new functional connection between Aβ and tau in AD. Here, we aimed to further elucidate the associations between hyperphosphorylated tau (tau), Aβ and pE-Aβ in human brain tissue. Methods: We examined 41 post mortem brains (63.4 % female, mean age 79.6 years, SE: ±1.5) of both AD (43.9%) and controls. Adjacent slides from frontal and entorhinal cortices were stained with AT8 (tau), 4G8 (Aβ) and pE-Aβ specific antibodies and the respective loads were assessed using image analysis. In a subset of cases western blot analysis was performed additionally. Results: Stepwise linear regression analysis with AT8 load as dependent variable and both 4G8 and pE-Aβ loads as independent variables revealed that pE-Aβ load but not 4G8 significantly predicted AT8 in frontal and entorhinal cortices. In the frontal cortex, only pE-Aβ independently correlated with both neuritic Braak stages and Thal Aβ stages, while in the entorhinal cortex only AT8 was correlated with neuritic Braak stages. Conclusions: We found a strong association between pE-Aβ and tau in human brain tissue. However, while pE-Aβ might initially trigger frontal tau accumulation other mechanisms seem to be responsible for initial entorhinal tau accumulation. Taken together our findings further strengthen the assumption of a functional connection between pE-Aβ and tau in AD.

P2-059 WITHDRAWN

P2-060 ANTI-AGGREGATION EFFECT OF AA-PEPTIDE ON BETA-AMYLOID PEPTIDES IN ALZHEIMER’S DISEASE

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Background: Since the development of research in Alzheimer’s diseases in 1990s, the disease was discovered close related to endogenous β-Amyloid peptides, especially aggregated oligomers and fibrils of which neurotoxicity has been confirmed. Therefore, Inhibition of Aβ, instead of blocking their production (e.g. secretase inhibitors), is rather promising in pharmaceutical research in AD. However, the identification of short sequences that effectively inhibit or prevent aggregation is still challenging. Taking advantage of the combinatorial chemistry, we designed and screened 400,000 of N-Acetylated-N-Aminoethyl peptides (AA peptide, unnatural peptidomimetics developed by our group) targeting the anti-aggregation effect of β-amyloid peptide. In this study, both the effect of anti-aggregation of