P4-152 PATHOLOGICAL TAU FROM ALZHEIMER'S BRAIN INDUCES SITE-SPECIFIC HYPERPHOSPHORYLATION AND SDS- AND REDUCING AGENT-RESISTANT HIGH MOLECULAR WEIGHT AGGREGATION OF TAU IN VIVO

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Background: Neurofibrillary tangles (NFTs) made up of hyperphosphorylated tau are the hallmark of Alzheimer's disease (AD) and related tauopathies. Hyperphosphorylation of tau is responsible for its loss of function, gain of toxicity and its aggregation to form NFTs. Injection of misfolded tau seeds into mouse brain induces phosphorylated tau aggregation, but the nature of tau phosphorylation in pathologic tau seeded pathology is unclear. **Methods:** Hyperphosphorylated and oligomeric tau isolated from AD brain (AD P-tau) was injected unilaterally into hippocampus of the human tau transgenic mice and tau knockout mice. Tau phosphorylation, the expression of tau kinases and tau phosphatases in the hippocampi and cortices were analyzed by Western blots. **Results:** We found that in addition to tau pathology, AD P-tau induced hyperphosphorylation of tau at Ser202/Thr205, Thr212, Ser214, Thr217, Ser262, and Ser422 in the ipsilateral hippocampus and at Ser422 in the contralateral hippocampus and in the ipsilateral cortex. AD P-tau induced AD-like high molecular weight aggregates of tau that was SDS- and reducing agent-resistant in the injected hippocampus. There were no detectable alterations in levels of tau phosphatases or tau kinases in AD P-tau-injected brains. Furthermore, we found that hyperphosphorylated tau was easier to be captured by AD P-tau and that aggregated tau was more difficult to be dephosphorylated than non-aggregated tau by protein phosphatase 2A (PP2A). **Conclusions:** Based on these findings, we speculate that AD P-tau seeds capture hyperphosphorylated tau to form aggregation, which leads to resistance to dephosphorylation by PP2A, resulting in tau hyperphosphorylation and tau pathology.

P4-153 LOSS OF BILIVERDIN REDUCTASE-A FAVORS TAU HYPER-PHOSPHORYLATION IN ALZHEIMER DISEASE

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Background: Hyper-active GSK-3βfavors Tau phosphorylation during the progression of Alzheimer disease (AD). Akt is one of the main kinases inhibiting GSK-3$\beta$and its activation occurs in response to neurotoxic stimuli including, i.e., oxidative stress. Biliverdin reductase-A (BVR-A) is a scaffold protein favoring the Akt-mediated inhibition of GSK-3β. Reduced BVR-A levels along with increased oxidative stress were observed early in the hippocampus of 3xTg-AD mice (at 6 months), thus suggesting that loss of BVR-A could be a limiting factor in the oxidative stress-induced Akt-mediated inhibition of GSK-3βin AD. **Methods:** We evaluated changes of BVR-A, Akt, GSK-3β, oxidative stress and Tau phosphorylation levels: (a) in brain from young (6-months) and old (12-months) 3xTg-AD mice; and (b) in post-mortem inferior parietal lobule (IPL) samples from amnestic mild cognitive impairment (MCI), from AD and from age-matched controls. Furthermore, similar analyses were performed *in vitro* in cells lacking BVR-A and treated with H_2O_2. **Results:** Reduced BVR-A levels along with: (a) increased oxidative stress; (b) reduced GSK-3βinhibition; and (c) increased Tau Ser404 phosphorylation (target of GSK-3βactivity) without changes of Akt activation in young mice, were observed. Similar findings were obtained in MCI, consistent with the notion that this is a molecular mechanism disrupted in humans. Interestingly, cells lacking BVR-A and treated with H_2O_2 showed reduced GSK-3βinhibition and increased Tau Ser404 phosphorylation, which resulted from a defect of Akt and GSK-3βphysical interaction. Reduced levels of Akt/GSK-3βcomplex were confirmed in bothyoung 3xTg-AD and MCI brain. **Conclusions:** We demonstrated that loss of BVR-A impairs the neuroprotective Akt-mediated inhibition of GSK-3βin response to oxidative stress, thus contributing to Tau hyper-phosphorylation in early stage AD. Such changes potential provides promising therapeutic targets for this devastating disorder.

P4-154 THE PROTEIN QUALITY CONTROL PROTEIN, UBIQUILIN-2, REGULATES TAU ACCUMULATION

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Background: Ubiquilin-2 (UBQLN2) is a protein quality control protein involved in shuttling ubiquitinated substrates to the proteasome for degradation and modulating autophagy. UBQLN2 has been implicated in neurodegenerative disease due to its accumulation in neuropathological deposits and its potential role in regulating protein dyshomeostasis common across different neurodegenerative disorders. The relationship of UBQLN2 to one of the most common aggregating proteins in disease, tau, is unknown. **Methods:** To determine the relevance of UBQLN2 dysregulation to human disease, we measured levels of soluble and insoluble UBQLN2 in human tauopathy brain tissue. To evaluate whether UBQLN2 regulates tau clearance, we first assessed levels of tau in human embryonic kidney-293 cells with and without UBQLN2. The ability of UBQLN2 to regulate other common aggregation-prone disease proteins, including alpha-synuclein was also evaluated. To determine whether UBQLN2 acts on tau *in vivo,* tau transgenic mice were crossed with UBQLN2 transgenic and knockout mice. **Results:** Co-expressed UBQLN2 markedly lowered levels of tau. Conversely, knockdown of UBQLN2 significantly elevated levels of tau. In contrast, a highly homologous protein, UBQLN1, did not exhibit the same ability to decrease tau levels. UBQLN2 overexpression *in vivo* was associated with decreased levels of insoluble tau. The possibility that UBQLN2 also undergoes alterations in disease was evidenced by the fact

that UBQLN2 insolubility is elevated in brains with tau pathology. **Conclusions:** Our findings highlight a new role for UBQLN2—but not UBQLN1—in managing levels of tau and other neurodegenerative disease proteins in the brain. However, increased insoluble UBQLN2 correlated with pathological tau accumulation suggest that UBQLN2 may be dysfunctional in tauopathies due to its intrinsic aggregation or accumulation in disease aggregates.

P4-155 INCREASED TAU PROTEIN TO *MAPT* MRNA RATIOS AND DECREASED LEVELS OF TAU FRAGMENTATION IN BRAINS OF HUMAN A152T-*MAPT* CARRIERS WITH TAUOPATHIES

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Background: Mutations in the gene that encodes the microtubule-associated protein tau (*MAPT)* cause frontotemporal dementia spectrum disorders (FTDs) but not Alzheimer's disease (AD). However, the rare A152T-variant of tau increases the risk for both AD and FTDs by an odds ratio of roughly 2. Understanding the underlying mechanisms could identify pathogenic convergence points between these conditions. We previously showed that transgenic mice expressing A152T-variant human tau protein (hTau-A152T) in glutamatergic forebrain neurons had lower human *MAPT* mRNA levels than transgenic mice expressing comparable levels of wildtype human tau protein (hTau-WT). hTau-A152T mice also had lower cerebral levels of hTau fragments. In contrast, other groups reported higher levels of tau fragmentation in iPS cell-derived neurons from A152T carriers than non-carriers. Here, we assessed which of these models more accurately predicts *MAPT* expression and tau processing in the human condition. **Methods:** Frozen *postmortem* brain tissues from human A152T carriers (n = 15) and non-carriers (n = 17) matched for age, sex, and autopsy diagnosis (AD, FTLD, PSP, or CBD) were compared by western blot analysis and quantitative RT-PCR. **Results:** Across tauopathies, human carriers and non-carriers had similar levels of full-length tau protein in sarkosyl-soluble fractions. In contrast, *MAPT* mRNA levels were roughly 50% lower in carriers than non-carriers, indicating that carriers have an increased tau protein to *MAPT* mRNA ratio. Levels of tau fragments were also significantly lower in carriers than non-carriers. These results are remarkably similar to those we obtained in transgenic mice expressing *MAPT* cDNAs from a heterologous promoter (*EMBO Rep.* 17: 530–551). **Conclusions:** Taken together, our findings suggest that the A152T substitution interferes with the degradation of tau at the protein level and that the reduced *MAPT* mRNA levels in carriers reflect a compensatory adjustment in gene expression. Although we consider this interpretation most plausible, alternative mechanisms affecting gene expression or mRNA turnover have not yet been excluded. Ongoing studies aim to identify the mechanisms by which the A152T substitution reduces both tau fragmentation and *MAPT* mRNA levels and to elucidate the processes by which hTau-A152T increases the risk of diverse tauopathies. Supported by the Tau Consortium.

P4-156 DISCOVERY OF NOVEL SMALL MOLECULE THERAPEUTICS FOR ALZHIEMER'S DISEASE BY TARGETING STERIC ZIPPERS INVOLVED IN TAU AGGREGATION

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Background: Frontotemporal dementia and Alzheimer's disease (AD) are progressive neurodegenerative diseases characterized in part by aggregation of the tau protein. At the molecular and cellular levels the aggregation of tau leads to the formation of neurofibrillary tangles (NFTs) that cause a loss of neuronal connectivity, initiate neurodegeneration, and result in the cognitive decline that is the hallmark of the disease. Tau aggregation is dependent on two highly aggregation-prone hexapeptide segments or "steric zippers"–VQIVYK and VQIINK–that are located in the repeat domains in the tau protein. **Methods:** ADRx has used a structure-based approach to design and optimize novel peptidic Tau Aggregation Inhibitors (pTAI) that bind to these segments in their aggregation-competent conformation. Here we show that pTAIs prevent tau aggregation both in biochemical and cellular assays as well as in transgenic rTg4510 mice using a viral delivery approach. ADRx has leveraged this approach to develop a targeted competition assay to identify small molecule compounds that mimic the properties of pTAIs and allow a more facile development path for delivery as a therapeutic. **Results:** A high-throughput screen of over 200,000 compounds has successfully identified multiple chemotypes that selectively disrupt the interaction of a potent pTAI to aggregated Tau. Similar to the pTAIs, these small molecules prevent aggregation of tau in ThT-based and cellular reporter assays. The unique nature of this competition assay has identified molecules that promise a level of specificity and potency superior to aggregation inhibitors identified by other means. **Conclusions:** We believe that these represent an exciting new set of molecules that have the potential to become the first therapeutics specifically targeting steric zippers to prevent tau aggregation.

P4-157 ROLE OF FYN IN A MOUSE MODEL OF TAU P301L TAUOPATHY

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Background: Src family non-receptor tyrosine kinase Fyn has been implicated in neurodegeneration of Alzheimer's disease. We have shown that tau P301L has a higher binding affinity, than WT tau, to Fyn. However, the role of Fyn in mediating disease pathogeneses in tauopathies, where Abeta protein is absent, is still not very well understood. **Methods:** We have used the somatic brain transgenic technique to produce tauopathy mouse models where AAV vectors deliver human tau P301L to the brains of newborn Fyn KO and WT mice. Fyn KO mice with severe hydrocephalus were identified by MRI and not used for comparisons. Mice were harvested at 6 months, examining tau phosphorylation at S199/S202 and Y18. Behavioral studies were also performed, with un-injected Fyn