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Enrichment of novel Tau isoform with altered biochemical properties in Alzheimer's disease

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Aberrant splicing in sporadic Alzheimer's disease: Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by the presence of extracellular amyloid-β plaques and Tau-containing intracellular neurofibrillary tangles (Knopman et al., 2021). Cognitive decline in AD due to neuronal and synaptic loss is caused by the complex interplay between pathological amyloid-β and Tau species with the impaired innate clearance pathways. There are two types of AD based on disease aetiology (Knopman et al., 2021). In early-onset familial AD, patients are inherited with autosomal dominant mutations in either amyloid precursor protein (*APP*) or presenilin-1/2 (*PSEN1/2*) genes which lead to elevated production of amyloid-β (Lanoiselee et al., 2017). On the other hand, late-onset sporadic AD is characterized by high pathological heterogeneity with aging and genetic variations as the major disease risk factors (Knopman et al., 2021). Recent high-throughput results from longitudinal, epidemiologic studies such as the Religious Orders Study and the Memory and Aging Project showed that aberrant mRNA splicing may participate in the progression of sporadic AD (Raj et al., 2018). Increased intron retention, an alternative splicing event where introns are not removed from mature mRNA transcripts, has also been linked to aging brains and AD pathogenesis (Adusumalli et al., 2019; Garcia-Escudero et al., 2021; Ngian et al., 2022).

Hyperphosphorylated Tau and tauopathy are hallmarks of AD: Microtubule-associated protein Tau is encoded by a single gene whose pre-mRNA undergoes alternative splicing to produce six protein isoforms in the human brain (Gotz et al., 2019; Knopman et al., 2021; Moloney et al., 2021). Full-length Tau protein, which contains 441 amino acids (herein Tau441), plays crucial roles in promoting the assembly of microtubules and stabilizing their structures in the axon of the neurons (Knopman et al., 2021; Moloney et al., 2021). Tauopathy is defined as a group of human neurological disorders that have Tau inclusions in the neurons or glia (Gotz et al., 2019). In AD and other types of tauopathies, mutations and/or aberrant post-translational modifications like hyperphosphorylation cause Tau to aggregate into paired helical filaments, which then form neurofibrillary tangles in the neuronal soma, neuropil threads in the neurites or neuritic plaques surrounding the amyloid-β core (Gotz et al., 2019; Moloney et al., 2021). The degree of neurofibrillary tangles load is strongly correlated with the clinical symptoms of AD whereby increased cognitive impairment is often associated with the advanced stage of Tau pathology (Gotz et al., 2019). While detailed mechanisms underlying the interaction between amyloid-β and Tau remain to be elucidated, neuropathological and animal model data suggest a complex yet synergistic relationship between both in AD aetiology (Gotz et al., 2019; Knopman et al., 2021). Consistent with its central role in AD pathological continuum, changes in the level of phosphorylated Tau at threonine 181/217 in the blood plasma and cerebrospinal fluids have recently been used as a diagnostic predictor for AD (Knopman et al., 2021).

Novel truncated Tau species produced by intron retention: Analysis of dorsolateral prefrontal cortex Religious Orders Study and the Memory and Aging Project dataset (syn21088596) (Raj et al., 2018) using IRFinder (Wong et al., 2017) revealed statistically significant retention of intron 11 in *Tau* mRNA transcripts in female (control, n = 32; AD, n = 34), but not male (control, n = 35; AD, n = 36) AD patients when compared to healthy subjects (Ngian et al., 2022). Retention of intron 11 (chr17: 46014325-46018617, hg38) introduces 19 additional amino acids followed by a premature stop codon, leading to the translation of truncated protein (herein Tau11i) (**Figure 1A**). The presence of Tau11i protein in the human brain was investigated by generating specific antibodies that recognize this unique sequence of amino acids encoded by intron 11. Consistent with the increased retention of intron 11 of *Tau* mRNA in AD patients, there is a significant enrichment of Tau11i protein in AD lysates harvested from five different brain cortex regions when compared to healthy controls (Ngian et al., 2022; **Figure 1B**). The brain regions examined include the frontal lobe, parietal lobe, temporal lobe, amygdala, and hippocampus from seven pairs of healthy subjects and AD patients. It is highly plausible that the presence of canonical polyadenylation signal within intron 11 protects *Tau11i* mRNA transcripts against nonsense-mediated decay, which in turn facilitates protein translation (Garcia-Escudero et al., 2021). Interestingly, immunofluorescence staining of AD temporal lobe sections showed that Tau11i proteins form granular-like aggregates that co-localize weakly with Tau neurofibrillary tangles, suggesting that Tau11i might be involved in pre-tangle assembly (Moloney et al., 2021).

Altered biochemical properties of truncated Tau11i: Interestingly, Tau11i proteins exhibit several pathological-like biochemical properties when expressed in 293 human embryonic kidney cells and neuronal cell types (Gotz et al., 2019; Ngian et al., 2022). First, unlike monomeric full-length Tau441, truncated Tau11i proteins oligomerize to form high molecular weight species in 293 cells and neurons. This observation could be explained by the absence of the C-terminal domain which functions to inhibit Tau polymerization (Gotz et al., 2019). Second, both ectopically expressed and endogenous Tau11i proteins are significantly enriched in Sarkosyl-insoluble fraction in the neurons and AD hippocampus respectively when compared to Tau441. This suggests that Tau11i proteins undergo misfolding and aggregation since Sarkosyl is a mild ionic denaturing detergent capable of only solubilizing natively folded proteins. In line with this hypothesis, monitoring the level of transiently expressed protein over a week and cycloheximide assay showed that Tau11i exhibits a lower protein turnover rate in the cells when compared to Tau441. Third, Tau11i proteins have weaker binding affinity to microtubule networks in cultured human neurons when compared to Tau441, likely due to the loss of its microtubule-binding domain repeat four (**Figure 1B**). Although these biochemical changes suggest that Tau11i may be involved in AD pathogenesis, follow-up studies with different brain regions from larger AD cohorts will be needed to provide insights into the tissue-specific roles played by Tau11i in the different Braak stages of the AD continuum.

Mechanisms underlying sex-linked differential intron retention: Significantly increased retention of *Tau* intron 11 was unexpectedly observed only in the female but not male AD cohorts (Ngian et al., 2022). While it remains unclear what regulates differential splicing patterns of specific introns, the sex-linked effect might be explained by epigenetic mechanism (Wong et al., 2017) and the extensive crosstalk between Tau aggregates and spliceosome complexes (Hsieh et al., 2019; Lester et al., 2021). It was well-documented that the rate of transcription and RNA splicing is intricately co-regulated by the level of DNA methylation. For instance, during granulocyte differentiation, binding of MeCP2 to methylated DNA alters the rate of RNA polymerase II elongation and

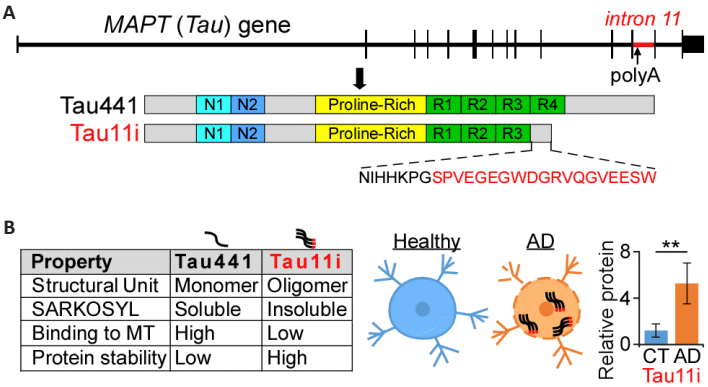

Figure 1 | **Retention of intron 11 leads to translation of novel truncated Tau11i isoform.**
(A) Schematic of human *MAPT* gene and the protein domains of different Tau isoforms. The 19 amino acids encoded by retained intron 11 are highlighted in red. (B) Compared to full-length Tau441, truncated Tau11i proteins form oligomers, are enriched in Sarkosyl-insoluble fraction, have weaker binding affinity to microtubule and higher protein stability in cells. Tau11i proteins form granular-like aggregates in the temporal lobes from AD patients. Immunoblot of the frontal lobe, temporal lobe, parietal lobe, amygdala, and hippocampus showed an elevated level of Tau11i protein in AD brain cortex tissues (n = 7, $^{**}P$ = 0.003, 2-tailed t-test). Adapted from Ngian et al. (2022). Created using CANVAS (version 12). AD: Alzheimer's disease; CT: healthy control; MAPT: microtubule-associated protein Tau; MT: microtubule; N1 and N2: amino-terminal inserts; polyA: polyadenylation signal; R1 to R4: microtubule-binding domain repeats; Tau11i: truncated Tau containing 19 amino acids encoded by retained intron 11; Tau441: full-length Tau with 441 amino acids.

facilitates the recruitment of splicing factors to inhibit aberrant intron retention (Wong et al., 2017). Thus, it is conceivable that the sex-specific DNA methylation patterns may contribute to the differential intron retention pattern between male and female cohorts (Ngian et al., 2022).

Accumulated evidence indicated that Tau aggregation is linked to aberrant RNA splicing during AD pathogenesis (Raj et al., 2018; Hsieh et al., 2019; Lester et al., 2021). Immunoprecipitation of soluble fractions from post-mortem human brains using Tau antibody followed with mass spectrometry analysis identified significant enrichment of spliceosome protein components like U1 small nuclear ribonucleoproteins U1-70K, SmD2, SmD3, SmN, and U1A, in the AD brain lysates when compared to non-demented control (Hsieh et al., 2019). Stratification of individual dorsolateral prefrontal cortex datapoint according to neurofibrillary tangles load revealed a significant association of cryptic splicing errors in mRNA transcripts with increasing Tau pathological burden in AD patients. In support of its causal role in RNA splicing, overexpression of Tau can alter the level of splicing machineries and splicing fidelity in Drosophila neurodegeneration model (Hsieh et al., 2019); and the differential splicing patterns in neurons that were derived from induced pluripotent stem cells (Raj et al., 2018).

Similar studies using brains from mouse models and dementia patients showed that cytosolic and nuclear Tau aggregates are enriched for small nuclear RNAs and small nucleolar RNAs, which regulate pre-mRNA splicing at nuclear speckles (Lester et al., 2021). Nuclear speckles are non-membranous organelles in the nucleus that contain assemblies of nascent RNA transcripts and components of the splicing machinery. Importantly, nuclear Tau aggregates colocalize with nuclear speckles, disrupting the composition, dynamics, and organization of their spliceosome components such as serine arginine repetitive matrix protein 2 (Lester et al., 2021).

More recently, incomplete X-chromosome inactivation in females was reported to cause elevated expression levels of sex-linked ubiquitin-specific peptidase 11 (*USP11*) gene (Yan et al., 2022). A higher level of USP11 detected in the female brain can deubiquitinate Tau at lysine 281, which in turn promotes acetylation and pathological accumulation of Tau protein (Yan et al., 2022). As such, it is plausible that the synergistic interaction between sex-specific epigenetic mechanisms and increased Tau pathology observed in women (Ngian et al., 2022; Yan et al., 2022) can lead to differential intron retention patterns at the *Tau* and perhaps other genes between male and female AD patients.

Unresolved role of Tau11i in AD pathogenesis: Beyond the altered biochemical properties, it is necessary to better understand how Tau11i protein may contribute to the continuum of AD pathogenesis. Neurofibrillary tangles develop through three defined levels of maturity that mark the different AD pathological stages in the neurons as abnormal Tau accumulates (Moloney et al., 2021). At the pre-tangles level, abnormal Tau initially deposits as tiny granular-like accumulations on the peripheral of the nucleus. These granular aggregates subsequently form fibers which develop into mature tangles (paired helical filaments) and replace the cytoplasm. Following the disintegration of the affected neuron and nucleus, the remaining extracellular "ghost" tangles appeared more loosely bundled and eosinophilic (Moloney et al., 2021). Interestingly, Tau11i forms granular-like structures that surround the nuclei and colocalize weakly with Tau4R fibrils in AD temporal lobe sections. This suggests that Tau11i might mark the pre-tangles and remain as globular non-filamentous form. Correlating Tau11i protein expression and its localization patterns with Braak severity, degree of amyloid-β and synuclein pathologies and neuronal injury from larger AD cohorts with different levels of cognitive impairment will provide better insights into its physiological or pathological roles.

Concluding remarks: Aberrant RNA splicing has been implicated in AD susceptibility through transcriptomic analysis (Raj et al., 2018; Adusumalli et al., 2019) with differential intron retention patterns observed at *Tau* mRNA transcripts between healthy control and AD patients' brain samples (Raj et al., 2018; Adusumalli et al., 2019; Garcia-Escudero et al., 2021; Ngian et al., 2022). Translation of *Tau* mRNA transcript with retained intron suggests that aberrant RNA splicing may lead to altered proteomes during AD progression (Ngian et al., 2022). While retention of intron 12 leads to a truncated non-aggregative Tau isoform (Garcia-Escudero et al., 2021), Tau11i isoform identified in our study exhibits pathology-like biochemical properties and was found to be enriched in the limited number of AD brain cortex tissues tested as compared to healthy control (**Figure 1B**). It will be important to elucidate the mechanisms underlying these differential splicing events, how Tau11i proteins form pre-tangles, and their involvement in AD pathogenesis.

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