Communication

The MAPT Isoform 0N3R Is Essential for Human Brain Development and Intolerant to Haploinsufficiency – Loss of Function as Disease Mechanism for TAU Associated Disease

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The *MAPT* Isoform 0N3R is Essential for Human Brain Development and Intolerant to Haploinsufficiency–Loss of Function as Disease Mechanism for TAU Associated Disease

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Abstract: TAU is the main disease driver in Alzheimer's and many other sporadic and genetic tauopathies. TAU is differently spliced, the role of individual splice-isoforms unclear. Murine *Mapt*-KO mice are healthy, and protected from Alzheimer's, but no such case has been reported in humans. Using gnomad database, we here demonstrate that the only isoform expressed during fetal human brain development is intolerant to mutation, while other brain and peripheral nervous system TAU isoforms are dispensable. With TAU targeted therapies for Alzheimer's and other tauopathies on the rise, we caution that TAU is essential human brain development.

Keywords: TAU, MAPT, neurodevelopment, neurogenetic and neurodegenerative disease, isoform, disease mechanism, dementia

Introduction

TAU is a major microtubule associated protein (MAP), regulates microtubule stability in the human brain similar to the other major MAPs MAP1A, MAP1B, and MAP2. The TAU protein is a key driver of the neurodegeneration observed in Alzheimer's disease (AD), but a crucial role of TAU in human brain development has not been established. Alternative splicing of the TAU-encoding *MAPT* gene results in the expression of peripheral 'Big TAU' isoforms, and six human brain-specific isoforms, only one of which, the 0N3R-isoform, is expressed in the fetal brain ((Hefti et al., 2018)(Takuma et al., 2003)(Nunez and Fischer, 1997)(Fischer and Baas, 2020)(Georgieff et al., 1991)(Goedert et al., 2006)).

Murine *Mapt*-KO mice from different laboratories all resulted in viable, fertile and principally healthy mice, with only minor neurological deficits (reviewed in (Ke et al., 2012)). Additionally, human *MAPT*-mutations associated with TAU-related disease show increased aggregation propensity, a pleiotropy of mouse models expressing human mutant TAU (also and commonly in addition to endogenous mouse Tau) recapitulate human disease, but with obvious limitations (for critical discussion see (Sahara and Yanai, 2023)), both in agreement with a toxic gain of function (i.e., in aggregation propensity)-based disease mechanism, usually resulting in the presence of insoluble neurofibrillary tangles (NFTs), which disrupt neuronal function.

Apart from its microtubule binding function, TAU's non-canonical physiological and pathological functions are debated and still not fully elucidated. Besides its role in driving disease pathology in ageing-associated disease, the microtubule-associated protein TAU is a key regulator of axonal microtubule stability and therefore mediates axonal transport, growth, synapse formation, and other MT-associated processes (reviewed in e.g., in (Zimmer-Bensch and Zempel, 2021)). In the adult human brain, six TAU isoforms are expressed, originating from alternative splicing of exons 2, 3, and 10 of the MAPT gene (Andreadis, 2012; Goedert et al., 1989)). The isoforms differ in the number

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of N-terminal inserts (0N, 1N, or 2N) and the C-terminal repeat (R) number (3R or 4R). During development, the isoform composition of the human brain switches from exclusively 0N3R to the presence of larger TAU isoforms, and results finally in about equal ratios of 3R to 4R TAU isoforms (Bullmann et al., 2009; Trabzuni et al., 2012). Notably, in the adult human brain 1N-TAU isoforms (1N3R and 1N4R) account already for 50% of the whole Tauom, while 2N-TAU isoforms are the least expressed isoforms (5-10% of Tauom). The significance of the TAU isoform expression ratio for neuronal health is further underscored by mutations in the *MAPT* gene which affect the splicing of TAU isoforms: E.g. reduction of 3R vs. 4R-TAU expression is associated with frontotemporal dementia with parkinsonism-17 (FTDP-17) (Buee and Delacourte, 1999; Goedert et al., 1995). Tauopathies are classically also categorized by the isoforms present in NFTs: Only 4R TAU isoforms accumulate in progressive supranuclear palsy (PSP) and cortical basal degeneration (CBD) patients' brains, mainly 3R TAU isoforms are present in argyrophyil grain disease (AGD) and down syndrome (DS) accumulations, but NFTs positive for both, 3R and 4R TAU isoforms, can be found in the brains of AD patients. While the functions - in particular of the individual isoforms - are underexplored, all of this implies a major role of the brain-specific TAU isoforms in disease pathogenesis.

In contrast, peripheral TAU (called 'Big TAU' due to its size ~2-fold as big as the CNS-isoforms), is not associated with disease, and not expressed in the brain. It is also not expressed in the CNS, except for neurons that project to the PNS, like spinal motor neurons, retinal ganglion cells, and many cranial nerve neurons, all of which express Big TAU as they mature into adulthood). The region of Big TAU corresponding to exon 5 (classically 4a) has almost no homology to known proteins and almost no putative phosphorylation sites, suggesting that it arose evolutionarily from an intron of another protein. Big TAU has a lower propensity to form toxic aggregates and fibrils, in line with its lack of disease association compared to brain TAU (Fischer and Baas, 2020).

While GoF is an established disease mechanism for MAPT-related disease, LoF is not. Microdeletions only encompassing the gene *MAPT* have not been described. Microdeletion at 17q21.31 (where *MAPT* is located) associated with disease usually encompass also the gene *KANSL1*, haploinsufficiency or heterozygous pathogenic mutations of which are sufficient to cause Koolen-de-Vries Syndrome (KdVS), a rare intellectual disability (ID) syndrome with dysmorphic features (Koolen et al., 2006; Shaw-Smith et al., 2006). Notably, the severity of the phenotype of KdVS is reported to be similar in patients with a pathogenic SNV in *KANSL1* and in patients with a heterozygous deletion that includes *MAPT* (Koolen et al., 2016). This means that because i) *MAPT* heterozygous deletions do not add significantly to the intellectual disability of KdVS, ii) *MAPT* heterozygous deletions are in principle compatible with life, and iii) several murine *Mapt*-KO models are basically healthy (with the exception of mild neurological deficits, in one study even due to haploinsufficiency and lack of compensation via other MAPs) there was was up to now no hint towards intolerance against LoF of *MAPT*.

This was also in line with gnomad, a database with ~140.000 exomes and genomes and the predicted probability of intolerance to LoF (pLI) for the MANE transcript (transcript ENST00000262410.10, see (Morales et al., 2022) for description of MANE) of *MAPT* being 0.01 (ranging from 0, completely tolerant, to 1, completely intolerant) with v.2.1.1. Also using the very recent and significantly improved release of gnomAD v4.0.0, which includes exome and genome data from ~800,000 total individuals (Karczewski et al., 2020), we found again that overall *MAPT* pLI is 0.00, in line with the above mentioned evidence of MAPT being tolerant to LoF. The MANE transcript is a theoretical transcript, which is in case of MAPT, however for a neuronal/neurological protein biologically of little relevance, because it is possibly only expressed in adipose tissue but not in the CNS/brain (s. also above), and the closest neuronal protein correlate (Big TAU) is only expressed in the PNS and non-brain tissues (Fischer and Baas, 2020). In contrast, during human (and other mammalian species) brain development, the isoform exclusively expressed is 0N3R-TAU (transcript ENST00000334239.12). For this isoform, the pLI-score is 0.96 (hence close to 1, which would mean predicted to be intolerant to LoF). The pLI-score of the other CNS-isoforms (all of which are only expressed weeks to months after birth, depending on the species and the isoform), all of which are based on and contain completely the 0N3R-isoform (Fig. 1A), ranges between 0.95 for 0N4R-TAU,

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which only contains 1 exon more than 0N3R, and 0.05 for 2N4R, which contains 3 more exons than 0N3R TAU (Figure 1A and Table 1). This is in line with the scarcity of LoF mutations in the regions that make up 0N3R-TAU, with an observerd over expected (o/e) of 0.3, which demonstrate a strong natural selection against LoF mutations in 0N3R-TAU. This is in gross contrast to the LoF mutational load of e.g. exons 5 (traditionally 4a) and 7, which carry an abundance of LoF mutations, but are not present (spliced out) in the human brain isoforms (Fig1A,B). This clearly hints towards the 0N3R-TAU isoform, as the only TAU isoform present in the developing brain, being essential for proper brain development. Linear regression analysis of the 6 brain isoform and the MANE transcript suggests mutation intolerance of ~285aa of the 353aa of the 0N3R TAU isoform (Fig.1. This LoF-intolerance puts 0N3R-TAU in line with the other major brain MAPs (i.e. MAP1A, MAP1B, and MAP2), all of which are intolerant to LoF (with a pLI-score of 1). MAP1A and MAP2 are currently not associated with a human disease (possibly because of incompatibility with life), and MAP1B associated with i.a. Periventricular Nodular Heterotopia type 9 (PVNH9), which includes malformation of cortical development, typical of brain microtubule impairments. Of note, even homozygous knockouts for murine *Map1a* (Liu et al., 2015), *Map1b (Pangratz-Fuehrer et al., 2005)* or *Map2* (Teng et al., 2001) are viable and show no or little cortical developmental abnormalities. It is hence not surprising that murine *Mapt*-KO mice also show only subtle neurologic defects, yet human brain TAU protein could still be essential for proper human brain development.

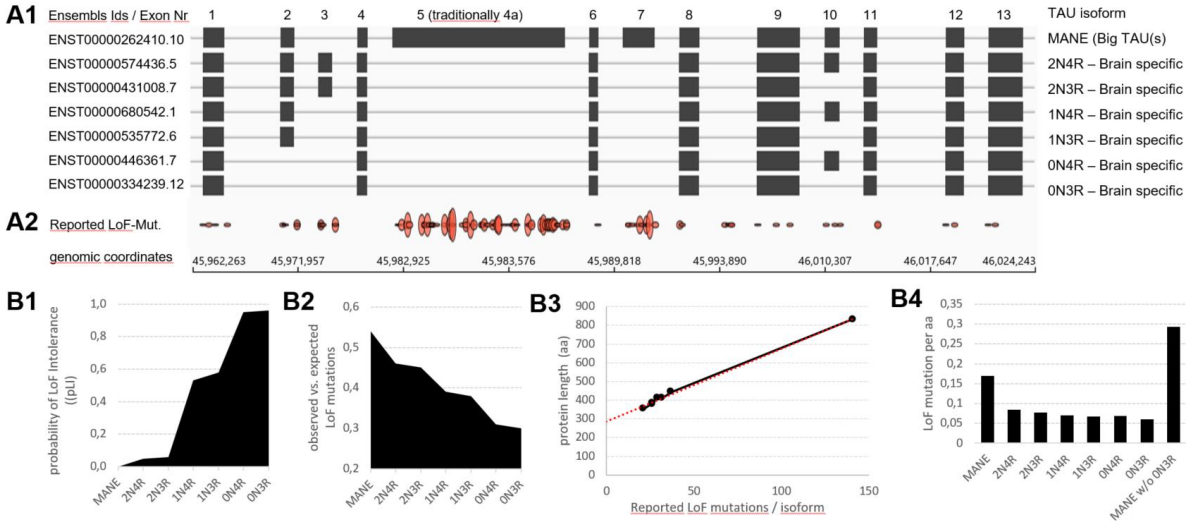

Figure 1. MAPT loss of function (LoF) mutations are underrepresented in the only TAU isoform present in the developing brain, but not in other brain isoforms or peripheral TAU. A: Graphical depiction (adapted from gnomad v4.0.0, selected isoforms shown) of (A1) the MANE transcript (upper row), the two most major isoforms (2N-TAUs, representing only ~10% of the TAUom of the brain, row 2 and 3), and the 4 main brain isoforms (rows 4-7), of which the 0N3R isoform is the only one present in the brain. Below (A2) are depicted the reported LoF mutations from ~725.000 NGS-datasets (exomes, only, unbiased selection, upper part), and the genomic coordinates (lower part) of chromosome 17. B1: Plots of the pLI score of the MANE-transcript (here representative for peripheral TAU) and the 6 individual brain isoforms. B2: Plots of the observed vs. expected number of LoF mutations in TAU. Note that the MANE transcript includes the other transcripts (s. A1). B3: Reported LoF-mutations vs. protein lengths and linear regression analysis indicate a ~300aa part of TAU were LoF mutations may not be tolerated. B4: LoF mutational load is ~3 fold higher in the MANE-transcript than in the 0N3R isoform, and ~5 higher in the MANE-transcript part without 0N3R-TAU exons. Note that 0N3R isoform is contained in the MANE-transcript. All data available at https://gnomad.broadinstitute.org/.

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Table 1. Table summarizing MANE-transcript (representative of peripheral TAU isoforms) properties and brain-specific isoforms for loss of function (LoF) mutations: Protein length (aa), probability of LoF-Intolerance (pLI), observed over expected LoF-Mutations, total LoF mutation load over the whole isoform, and LoF/aa (protein length).

TAU isoform	aa	pLI	LoF o/e	LoF-Mut load	LoF-Mut/aa
MANE	833	0.00	0.54 (0.41 - 0.72)	141	0.169268
2N4R	441	0.05	0.46 (0.31 - 0.68)	37	0.0839
2N3R	412	0.06	0.45 (0.31 - 0.68)	32	0.07767
1N4R	410	0.53	0.39 (0.26 - 0.61)	29	0.070732
1N3R	383	0.58	0.38 (0.25 - 0.61)	26	0.067885
0N4R	381	0.95	0.31 (0.19 - 0.53)	26	0.068241
0N3R	352	0.96	0.3 (0.18 - 0.53)	21	0.059659

We have recently created human *MAPT*-KO iPSCs using Crispr-Cas9and differentiated them into cortical neurons. In contrast to murine *Mapt*-KO primary neurons (which showed neurite outgrowth deficits only 1-2 days after plating, (Dawson et al., 2001)), human neurons devoid of TAU protein showed neurite outgrowth deficits after 7 days, shortening of the axon-initial-segment, and a strong trend to hyperexcitability. Using proteomics, RNAseq and immunostainings we could not identify compensatory upregulation of other MAPs. Reintroduction of any of the 6 human brain specific TAU isoforms could rescue these deficit (Buchholz et al, unpublished, see also (Buchholz et al., 2022)), indicative of the ability of all the isoform to compensate for each other at least in development. This could e.g. explain that splice mutations resulting in the presence of other brain isoform may well be tolerated, but not other LoF.

Conclusions

In combination with the data presented here, we reason that LoF mutations in the 0N3R isoform impacts human brain development sufficiently to result in negative selection, e.g. via ID. As *MAPT* deficiency does apparently not impact the severity of KdVS in humans, we must assume that LoF mutations in 0N3R-TAU results in mild-moderate intellectual impairments, and as it is significantly only expressed in brain, no dysmorphic features. As mice and human neurons deficient in TAU display hyperexcitability, at least increased susceptibility to epilepsy must be expected. We hence postulate a novel intellectual disability syndrome with mild-moderate disability and increased susceptibility to epilepsy, but no dysmorphic features.

This analysis, if true and confirmed by functional studies, would also imply that depletion (e.g. as would be the case in RNAi- or antibody based approaches for Alzheimer Disease and related tauopathies) of 0N3R-TAU may have subtle neurological side effects, as in humans the 0N3R-isoform is continuously expressed, and may be implicated in adult neurogenesis/neurodevelopment.

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