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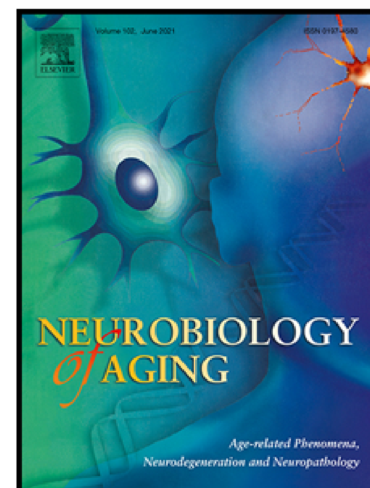

A clinical, molecular genetics and pathological study of a FTDP-17 family with a heterozygous splicing variant c.823-10G¿T at the intron 9/exon 10 of the MAPT gene

Diana A. Olszewska , Conor Fearon , Christopher McGuigan , Terri P McVeigh , Henry Houlden , James M Polke , Brian Lawlor , Robert Coen , Michael Hutchinson , Michael Hutton , Alan Beausang , Isabelle Delon , Francesca Brett , Ioanna Sevastou , Nuria Seto-Salvia , Rohan de Silva , Tim Lynch

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Highlights:

- c.823-10G>T Microtubule associated tau (*MAPT)* gene variant was reported in one family with frontotemporal dementia
- No pathological data was available confirming the disease.
- This is the first description of the pathology confirming that the variant is disease causing.
- This is the first description of the MND associated with c.823-10G>T *MAPT* variant.

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Authors:
Diana A. Olszewska, [1] Conor Fearon, [1] Christopher McGuigan, [2] Terri P McVeigh, [3] Henry Houlden, [4] James M Polke, [4] Brian Lawlor, [5] Robert Coen, [5] Michael Hutchinson, [2] Michael Hutton, [6] Alan Beausang, [7] Isabelle Delon, [8] Francesca Brett, [7] Ioanna Sevastou, [9] Nuria Seto-Salvia, [9] Rohan de Silva, [9] Tim Lynch. [1,10,11]

Affiliations:
1. Dublin Neurological Institute, (www.neurologicalinstitute.ie) and the Department of Neurology, Mater Misericordiae University Hospital, Dublin, Ireland,
2. Department of Neurology, St Vincent's University Hospital, Dublin, Ireland,
3. Our Lady's Children's Hospital, Crumlin, Dublin, Ireland.
4. Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, London WC1N 3BG, UK,
5. Mercer's Institute of Aging, St James's Hospital Dublin, Ireland,
6. Eli Lilly, Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, USA
7. Department of Neuropathology, Beaumont Hospital, Dublin, Ireland,
8. East Genomic Laboratory Hub, Cambridge University Hospital NHS Foundation Trust, Addenbrooke's Treatment Centre, Hills Road, Cambridge, CB2 0QQ, UK,
9. Reta Lila Weston Institute & Department of Clinical and Movement Neuroscience, UCL Queen Square Institute of Neurology, London WC1N 1PJ, UK,
10. Health affairs, University College Dublin, Dublin, Ireland,
11. Ireland East Hospital Group, Dublin, Ireland

Corresponding author:
Prof. Tim Lynch
The Dublin Neurological Institute at the Mater Misericordiae University Hospital
57 Eccless Street,
Dublin, Ireland.
email: tlynch@dni.ie

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Abstract:

We report the first clinical-radiological-genetic-molecular-pathological study of a kindred with c.823-10G>T *MAPT* intronic variant (rs63749974) associated with frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17). We describe the clinical spectrum within this family and emphasize the association between *MAPT* gene variants and

motor neuron disease. This report of a second family with FTDP-17 associated with c.823-10G>T *MAPT* variant strongly supports pathogenicity of the variant and confirms it is a 4-repeat (4R) tauopathy. This intronic point mutation, probably strengthens the polypyrimidine tract and alters the splicing of exon 10 (10 nucleotides into intron 9) close to the 3' splice site.

Keywords: c.823-10G>T, intron 9/exon 10 mutation, frontotemporal dementia, genetics, neuropathology

List of abbreviations:

ACMG: The American College of Medical Genetics and Genomics

DDPAC: disinhibition-dementia-parkinsonism-amyotrophy-complex

Ex: exon

FWD: forward

GFAP: glial fibrillary acidic protein

GnomAD: Genome Aggregation Database

H&E: Hematoxylin and eosin

HGMD: The Human Gene Mutation Database

MAPT: microtubule-associated protein tau

MMSE: Mini–Mental State Examination

MoCA: Montreal Cognitive Assessment Score (MoCA)

MSA-C: Multiple system atrophy cerebellar subtype

NNSPLICE: Splice Site Prediction by Neural Network-NNSPLICE

R: repeat

R1, 2, 3: brain region 1, 2, 3

RT-PCR: Reverse transcription polymerase chain reaction

RWS: reverse

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SSF: Splice Site Finder

WMS-R: Wechsler Memory Scale-Revised

1. Introduction:

In 1994, we reported an Irish-American family with frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) and termed this disorder disinhibition-dementia-parkinsonism-amyotrophy-complex (DDPAC) (Lynch et al., 1994; Wilhelmsen et al., 1994; Sima et al., 1996). This led to a clinical-radiological-pathological-genetic linkage study and ultimate cloning of the microtubule-associated protein tau gene (*MAPT*) on chromosome 17q21.31 (Hutton et al., 1998; Spillantini et al., 1998). Variants in *MAPT* are responsible for 15-20% of hereditary frontotemporal lobar degeneration (FTLD). Other common genetic causes of FTLD include progranulin *(GRN)* pathogenic variants (20-25% of FTLD) and hexanucleotide expansion repeats in the open reading frame of chromosome 9 (*C9orf72*) gene (3-48% of FTLD depending on the report) (DeJesus-Hernandez M et al., 2011, Majounie E et al. 2012, McCarthy et al. 2015). Of the genetic causes of FTLD, *C9orf72,* is most commonly associated with motor neuron disease (MND) (van Blitterswijk et al. 2013). However, as indicated by "amyotrophy" in DDPAC, *MAPT* variants are associated with later-onset MND (Moore et al., 2020).

Fifty-four pathogenic variants (coding, missense, deletions and intronic) (some close to the splice-donor site 3' of the alternatively spliced exon 10), of *MAPT* have been identified (McCarthy et al., 2015). In 1998 we (Hutton et al., 1998) and others (Spillantini et al., 1998) identified the +14 C>T exon 10/intron 10 boundary *MAPT* variant in the DDPAC family. In 2015, we "closed the tau stem loop" by describing the 'missing' c.915+15A>C variant at the intronic boundary downstream of exon 10 (note that *MAPT* variants are described in this

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report according to RefSeq transcript NM_005910.5 – c.823-915 encodes exon 9 of this transcript, though it is commonly referred to as 'exon 10' of the *MAPT* gene according to an alternative exon numbering convention), in another Irish FTDP-17 family (Grover et al., 1999; Qian and Liu, 2014; McCarthy et al. 2015).

In 2006, the c.823-10G>T *MAPT* variant was reported in one FTDP-17 family (Malkani *et al*, 2006). However, it remained undetermined whether c.823-10G>T is a pathogenic or a benign intronic variant as there was no pathological proof of FTLD, nor was there molecular confirmation of a 4R tauopathy. There have been no subsequent reports of other FTDP-17 patient/families associated with this *MAPT* intronic variant. We now present a clinical-radiological-genetic-molecular-pathological study of an Irish FTLD family associated with the c.823-10G>T variant. Our kindred is the second family with c.823-10G>T variant co-segregating with FTDP-17. We present the first pathological study of a c.823-10G>T variant family, confirming that this variant results in altered *MAPT* exon 10 splicing leading to increased 4R tau and provide supportive evidence of pathogenicity of c.823-10G>T.

2. Materials & Methods:

Mater Misericordiae University Hospital, Dublin granted the ethical approval. Informed consent was obtained. We conducted a detailed family study including chart review of family members and neurological examination of the proband (Fig. 1A III-1) and two siblings (Fig. 1A III-5, IIII-7). MRI brain imaging (Suppl. Fig. 1) of the proband (Fig. 1A III-1), genetic analysis of blood samples from the proband (Suppl. Fig. 1) and two unaffected siblings and necropsy of the proband (Fig. 1A III-1) and his brother (Fig.1A III-4) were performed (Fig. 3). The brain tissue was analysed using RT-PCR and Western blot (Fig. 4).

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2.1 DNA sequencing:

We performed Sanger sequencing using standard techniques and Mutation Surveyor software of exons 1-13 and intron boundaries of the *MAPT* gene (excluding exons 4a, 6, 8 which are not usually transcribed in the 6 major brain isoforms in the adult brain). The PCR primers used for exon 10 were FWD: 5'-AGGGTGGCGCATGTCACT-3'; RVS: 5'-CCCAAGAAGGATTTATTCTATGC-3', other primer sequences are available on request. The results were classified as per the American College of Medical Genetics and Genomics (ACMG) guidelines (Richards et al. 2015).

2.2 RT-PCR analysis:

RNA was isolated from 3 brain sections (inferior parietal, mid striatum, thalamus) from the proband (Fig 1A. III-1) and control brains from a patient with a multiple system atrophy cerebellar subtype (MSA-C) and a patient with Pick's disease (PiD) using TRIzol™ (Life Technologies) and cDNA derived using Superscript III First-Strand Kit (Invitrogen). For PCR analysis of *MAPT* exon 10 splicing, we used primers from flanking exons: Forward (exon 9): 5'-GTCAAGTCCAAGATCGGCTC-3' and Reverse (exon 13): 5'-TGGTCTGTCTTGGCTTTGGC-3'(Rodriguez-Martin et al. 2005). On agarose gels, we detected an intermediate band of approximately 380bp between those for the 3R and 4R-tau mRNA bands (305 bp and 397bp, respectively) (Fig. 4). To resolve this, we used the fluorescent FAM-labelled forward primer with capillary electrophoresis and confirmed that this is a heteroduplex artefact, as previously described (Grover et al.1999; Kalbfuss et al. 2001).

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2.3 Haplotype analysis:

Additionally, we determined the *MAPT* haplotype in the proband, and his two unaffected siblings by testing for the presence of a 238–base pair deletion, characteristic of the H2 haplotype, located between exons 9 and 10 (Baker et al 1999). Genomic DNA was extracted from blood using standard techniques. The primers used were: Forward 5'-GGAAGACGTTCTCACTGATCTG-3' and Reverse 5'-AGGAGTCTGGCTTCAGTCTCTC-3'. The analysis was performed using previous standard protocols (Sposito et al 2015). Two controls (H1/H1 and H1/H2) were included to confirm the PCR was optimized for the detection of both haplotypes.

2.4 Western blot analysis:

Sarkosyl-insoluble tau from the same brain sections was isolated as previously described (Goedert et al 1992). Briefly, frozen brain was homogenized in buffer (10mM Tris-HCl, pH=7.5, 0.8M NaCl, 1mM EGTA and 10% sucrose (10x vol (w/v)) and centrifuged for 10 min at 16,000 xg. After retaining supernatant, the pellet was re-homogenised in in 5x vol of homogenization buffer and re-centrifuged and supernatants pooled. N-lauroylsarcosinate (w/v; Sigma-Aldrich) was added to 1% (w/v) and samples rocked for 1h at room temperature. Sarkosyl-insoluble pellets were then obtained by centrifugation at 100,000 x g for 1h and resuspended in 50mM Tris-HCl buffer (pH=7.5), 0.2ml per gm starting material. For dephosphorylation, pellets were then solubilized in 4M guanidium hydrochloride with shaking for 1h at room temperature. Guanidium hydrochloride was then removed by dialysis into 50mM Tris-HCl pH=7.5 buffer followed by dephosphorylation with 20U/µl λ-phosphatase (New England Biolabs) for 3h at 30°C (Hanger et al. 2002). Dephosphoryated

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samples were then analysed by Western blot and tau detected with rabbit anti-tau polyclonal antibody (Dako; 1/10,000) and IRDye-800CW secondary antibody (Li-Cor) with imaging on on a Li-Cor Odyssey Fc.

Data availability:

All data pertaining to this manuscript will be available upon reasonable request.

3. Results:

3.1 Case series report:

The proband (Fig. 1A. III-1) was an Irish man who ceased working as a cameraman aged 50 because of poor concentration. He became distant and laughed inappropriately. Aged 54 he developed tremor, stiffness and frequent falls followed by depression (age 60). He was diagnosed with FTD at 61. He was admitted to a nursing home and became wheelchair-dependent and anarthric. He had little response to Levodopa/Carbidopa. At age 61 his Montreal Cognitive Assessment (MoCA) test was 16/30. His cerebrospinal fluid (CSF) analysis showed slightly increased albumin 0.246 g/l (0.140-0.200), slightly low IgG at 0.017g/l (0.02-0.04), but normal IgG index 0.480 (0.0-0.7). CSF tau and amyloid were not studied. He died aged 65 and necropsy was performed (Fig. 3).

The proband had four brothers and two sisters (Fig. 1A), three of whom had early-onset dementia with a similar phenotype (Suppl. Table 1). One of the proband's brothers (Fig. 1A: III-2) became "odd" in his forties, e.g. eating a half-eaten apple from a bin and making up stories. He was aggressive, "bossy", intense and forgetful. He was diagnosed with FTD and died at a nursing home aged 59.

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Another brother (Fig. 1A:III-4) also developed early-onset dementia (Suppl. Table 1). He became “odd” in his forties, paranoid and withdrawn. He indicated, “People feel I am like my brother” (Fig. 1A III-2). In his early fifties he failed to recognise familiar friends and was inattentive with personal hygene. Parkinsonism was an early feature and fasciculations of face and trunk were noticed terminally. Aged 52, he scored 27/30 on a Mini-Mental State Examination (MMSE) decreasing to 22/30 eleven months later (Suppl. Table 2 additional testing). At age 53, he was admitted to a hospital with confusion, urinary incontinence and decreased consciousness. He developed type II respiratory failure requiring intubation and ventilation for apnoeic episodes. Fasciculations around the shoulders were detected and he was diagnosed with MND. He deteriorated, died from pneumonia and underwent autopsy.

The proband’s sister (Fig. 1A: III-6) became inappropriate and vulgar in public aged 50. She was impatient, forgetful and less talkative. She sometimes used the wrong word. She stopped socialising and often wore the same outfit daily. She developed a “sweet tooth” and became obsessive about security at home. She scored 21/30 on her MMSE (points lost on orientation and recall) and had difficulty performing the Luria test. She described the cookie-jar picture in short hesitant sentences. She had difficulty naming faces but identified a United States President as such (Nixon). She described a pyramid as “a roof”, and an igloo “a little cave”. She had a monotonous voice, masked facies, slow smile and slow left shoulder shrug. There was mild rigidity, slower rapid alternating movements and decreased left arm swing. She died aged 61.

Three other siblings are not clinically affected. An unaffected brother (Fig. 1A:III-3) died from a lung cancer aged 49. Two other siblings are well including a 62-year-old brother (Fig. 1A:III-5) and a 77-year-old sister (Fig. 1A:III-7). Both had a normal neurological examination (except for bilateral postural and kinetic hand tremor in the brother). The brother scored 27/30 on MoCA and 16/18 Frontotemporal Assessment Battery (FAB) testing (sister

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MoCA 26/30, FAB 14/18). The unaffected siblings would be expected to have symptoms by now considering their age and a fully penetrant disorder.

The proband's father (Fig.1A:II-7) became short-tempered and forgetful aged 43. During his daughter's wedding he asked, "is this the chap she is getting married to". He kept sweets in his pocket, was "childish", depressed, made mistakes in his job, and attended multiple Sunday church services. He died aged 53. The proband's mother (Fig. 1: II-8) developed dementia in the late 70s and died at 88. A necropsy was not carried out. A maternal aunt (Fig. 1A: II-10) developed dementia and died in a nursing home.

3.2 Neuroimaging:

The proband's MRI brain (Fig. 1A: III-1), (>10 years after onset), demonstrated severe bitemporal/moderate bifrontal atrophy with relative sparing of parieto-occipital lobes (Suppl. Fig. 1), atrophic corpus callosum and hydrocephalous ex-vacuo.

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3.3 Neuropathological examination:

3.3.1 Proband (Fig. 1A. III-1)

Macroscopic description: There was severe atrophy of frontal and temporal lobes with normal cortical ribbon throughout. Axial brainstem sections showed a pale substantia nigra.

Microscopy: There was marked cortical spongiosis and neuronal loss most marked in the superficial cortical laminae of the frontal, temporal lobes, insular cortex, anterior cingulate and less prominent in the parietal lobes and absent in the occipital lobes. Many of the residual cortical neurons were "ballooned neurons". No Pick body-like inclusions were seen. The corpus callosum and subcortical/periventricular white matter showed secondary degeneration.

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The midbrain (level of the red nucleus) showed loss of nigral pigmented neurons with pigmentary incontinence.

Frequent tau-immunopositive (AT8 monoclonal mouse anti-human PHF-tau pS202/pT205, Clone AT8, Innogen, dilution 1/2000) neuropil threads and neuronal inclusions were present in the cerebral cortex (frontal, temporal lobes, to a lesser extent in parietal and sparse in occipital lobes), basal ganglia, diencephalon, brainstem and dentate. Spherical tau-immunopositive inclusions were very frequent in the dentate fascia, amygdala, red nucleus, substantia nigra, periaqueductal grey matter and superior colliculus, the tegmentum, basis pontis and medulla. There was marked granular staining throughout the white matter and extremely frequent perinuclear cytoplasmic tau aggregations within oligodendrocytes. Three-repeat (3R)-tau staining (RD3 anti-tau monoclonal antibody, clone 8E6/C11, (Millipore) (deSilva et al. 2003) showed occasional positive neuronal cytoplasmic inclusions, glial cytoplasmic inclusions and dystrophic neurites in the cerebral hemispheres, anterior striatum and midbrain. Four-repeat (4R)-tau staining (RD3 anti-tau monoclonal antibody, clone 1E1/A6, (Millipore) (deSilva et al. 2003) revealed abundant neuronal cytoplasmic inclusions, glial cytoplasmic inclusions and dystrophic neurites in the same areas.

The features show 4R tauopathy and are consistent with those described in FTDP-17 (Fig. 3) (Sima et al. 1996).

3.3.2 The proband's brother (Fig. 1A:III-4)

Macroscopic examination: There was no disproportionate frontal atrophy; however, there was hippocampal and temporal horn atrophy, together with asymmetry of spinal cord.

Microscopy: Spongiosis (laminae 1-3) and mild-moderate astrocytic gliosis affecting temporal lobes more than frontal lobes were seen with similar changes in the hippocampus,

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amygdala, caudate, globus pallidus, substantia nigra and locus coeruleus. Occipital lobe was normal on H&E. There was anterior horn cell loss with gliosis in the spinal cord (cervical- and thoracic-predominant). 3R tau staining showed occasional positive inclusions. 4R tau staining demonstrated inclusions in the parahippocampal gyrus, both in astrocytes and neurons in the substantia nigra and locus coeruleus but no inclusions in the cerebellum or spinal cord. TDP-43 staining showed no evidence of accumulation in the cytoplasm and normal neuronal staining. Lewy bodies, neurofibrillary tangles and Pick body-like inclusions were absent. Thus, tau antibody staining for RD4/PHF confirm this as a 4R tauopathy (Fig. 3 I-K).

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3.4 Muscle biopsies at autopsy (deltoid and quadriceps):

There were angular atrophic fibres scattered among fibres of normal size. Routine ATPase preparations showed type-1 and type-2 fibre atrophy with evidence of fibre-type grouping (deltoid>quadriceps), consistent with deltoid and quadriceps denervation.

3.5 Genetic and Segregation analysis results:

Sanger sequencing showed proband's (Fig. 1A. III-1) heterozygosity for the splicing variant c.823-10G>T at the intron 9/exon 10 boundary of the *MAPT* gene (Fig. IB and Fig. 2) not detected in the two asymptomatic siblings (III-5 and III-7).

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3.6 RT-PCR and Western blot results:

In order to assess the effect of the c.823-10G>T mutation on alternative splicing of *MAPT* exon 10, we used RT-PCR with primers in flanking exons 9 and 13. Compared to the controls, the proband's brain showed an almost complete shift to exon 10-containing (4R-tau) transcript, particularly in the inferior parietal and mid striatum regions where the exon 10^- (3R-tau) band is scarcely detectable. As expected, with a control MSA-C brain, we show equal ratio of exon 10^+/exon 10^- (4R/3R-tau), and with a PiD brain, we show clearly increased exon 10^- (3R) tau (Fig. 4).

This strong shift in splicing of exon 10 is clearly reflected in the sarkosyl-insoluble pathological tau, with the proband almost exclusively displaying 4R-tau, particularly the 0N4R isoform (Fig. 4). The above MSA-C case showed equal 3R/4R-tau isoform ratios and the PiD case (PiD1) showed similar isoform ratios to MSA-C. A second PiD case (PiD2) had a corresponding, strong shift to elevated 3R-tau comprising mainly of 0N3R and 1N3R isoforms (Fig. 4). These data show that like other *MAPT* exon 10 splicing mutations, the c.823-10G>T mutation causes a 4R-tauopathy by increased exon 10 splicing with elevated levels of the more fibrillogenic 4R-tau (Fig. 4).

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3.7 The haplotype analysis results:

The haplotype analysis showed that the proband (III-1) was an H1 homozygote, and the unaffected siblings (III-5, III-7) were both H1H2 heterozygotes. The result, demonstrating that the variant was on the H1 background.

4 Discussion:

In the adult human brain, there are six tau isoforms distinguished by the presence or absence of 29 and 58 amino acids in the *MAPT* amino-terminal half and a 31 amino-acid repeat domain in the *MAPT* carboxy-terminal half encoded by exon 10 (Goedert et al. 1989, Spillantini et al. 1997, Hasegawa et al. 1998, Hong et al. 1998). The 31 amino-acid repeat domain is included in tau isoforms with four microtubule-binding repeats (4R-tau). In the healthy adult brain, there is approximately an equal 3R-/4R_tau ratio (Malkani et al. 2006, AD/FTD database). Multiple exonic and intronic variants within and around exon 10 impact splicing and lead to increased production of exon 10-containing transcripts (Hutton et al. 1998), thus resulting in an increased ratio of 4R- to 3R- tau isoforms (Hutton et al. 1998). 4R-tau isoforms bind microtubules more robust and produce greater levels of microtubule assembly *in vitro* compared to 3R-tau isoforms (Strang et al. 2019). However, overproduction of the more "aggregation-prone" 4R-tau may lead to the eventual assembly of 4R-tau into toxic aggregates including the wide twisted ribbons observed in several families with these *MAPT* variants (Spillantini et al. 1997). There are two major non-recombining *MAPT* haplotypes, H1 and H2, defined by a region of linkage disequilibrium of over 900 kb (Pastor et al. 2002). Both haplotypes influence *MAPT* expression and splicing and studies have established the overrepresentation of H1 haplotype in 4-repeat tauopathies such as progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD) (Pastor et al. 2002). However, data in frontotemporal dementia as the group are conflicting (Dilliot et al. 2019).

The exon 10 intronic variants are located around the splice acceptor and donor sites. Variants on the 3' end of exon 10 (5' splice site), include c.915+3G>A, c.915+12C>T, c.915+13A>G, c.915+14C>T, c.915+15A>C and c.915+16C>T (Hutton et al. 1998, Varani et al. 1999,

Yasuda et al. 2000, Miyamoto et al. 2001, McCarthy et al. 2015). A transgenic mouse expressing human *MAPT* carrying the c.915+16C>T variant has increased 4R-tau and tau pathology confirming that overproduction of 4R-tau leads to the development of tauopathies (Umeda et al. 2013).

The c.823-10G>T (-10) mutation, described here, was previously reported by Malkani et al. (2006) in the intronic region immediately upstream of exon 10. It is predicted to strengthen the polypyrimidine tract thus affecting the splicing of exon 10 close to the 3' splice site (Malkani et al. 2016). The mutation replaces purine with pyrimidine which is sufficient to enhance exon inclusion to near-constitutive levels. This expands the range of mechanisms by which pathogenic mutations are known to increase *MAPT* exon 10 splicing and thus increasing levels of 4R-tau. Remarkably only one exonic mutation (delK280) has been shown to have the opposite effect of reducing exon 10 incorporation and increasing 3R-tau, this is despite the multitude of positions at which mutations could occur that would cause reduced splicing (van Swieten et al. 2007). Moreover, interpretation of the delK280 mutation effect on splicing is complicated by the amino acid sequence change that also occurs with this mutation and which is known to dramatically reduce microtubule binding (van Swieten et al. 2007). In addition, in concert with the report by Malkani and colleagues (Malkani et al. 2006), we show that c.823-10G>T variant is on the H1 allele which presents the possibility of a common founder. Overall our findings add further support for a pathogenic model for the exon 10 splicing mutations where the absolute increase in 4R-tau and its associated consequences, most obviously increased aggregation, that ultimately cause FTDP-17 rather than a shift in the 4R:3R tau ratio.

In our family the proband's brother (III-4) was admitted with type II respiratory failure with periods of apnoea and fasciculations leading to a diagnosis of MND. The necropsy did not show diaphragmatic denervation, but demonstrated anterior horn cell loss, more severe in the

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cervical and thoracic region. This is a very similar observation to the pathology found in the DDPAC family where the two spinal cords studied had anterior horn cell loss and one of the patients had fasciculations, muscle wasting, weakness, clonus and diffuse denervation on EMG (Lynch et al. 1994). This same patient developed a central hypoventilation syndrome requiring tracheostomy and phrenic nerve pacemaker (Ondine's curse) (Lynch et al. 1994).

As mentioned earlier, *C9orf72* is the most common cause of FTD associated with amyotrophic lateral sclerosis (FTD-ALS), reported by Moore and colleagues (Moore et al. 2020) in 11.1% (n=157) of 1433 *C9orf72* carriers and ALS alone in 19.3% (n=276). *MAPT* gene associations with both FTD and ALS is much rarer, however, it occurs (FTD-ALS: 0.3% (2/279) and ALS: 0.1% (1/791)) especially with later development of ALS in patients with FTD (Lynch et al. 1994). *MAPT* variants in FTD and ALS should be kept in mind in *C9orf72*-negative patients (Moore et al. 2020).

The proband from the first FTDP-17 family reported with the c.823-10G>T *MAPT* variant that co-segregated with the disease was a 55-year-old man with progressive memory loss, outbursts of anger, emotional lability and disinhibition (Malkani et al. 2006). He had no parkinsonism, and his brain MRI was normal. The proband's sister was a 45-year-old-woman who spent money compulsively from the age of 38. She was obsessed with house chores and developed memory loss and hypophonia. The proband's father had memory problems from age 41, anger outbursts and poor social skills. He was in a nursing home from age 44 and died aged 50. However, there was no neuropathological data available from this pedigree to confirm the diagnosis of FTLD (Malkani et al. 2006, AD/FTD database, Maffucci et al. 2019). There has been no other patient reported since 2006 to confirm or refute c.823-10G>T as a causative *MAPT* variant of FTDP-17.

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Segregation information in our family (2 informative meioses) and in the previously reported family (3 informative meioses) (Malkani et al. 2006) (ACMG PP1-2 families) (Richards et al. 2015) is strongly supportive of pathogenicity (Moore et al. 2020). This finding, in combination with a supportive phenotype of 4R tauopathy at autopsy (ACMG PP4) (Richards et al. 2015), and functional studies (ACMG PS3) support the premise that c.823-10G>T *MAPT* variant is pathogenic. Other supporting factors include the location of the variant in relation to the polypyrimidine tract in intron 9 (*in sillico* prediction software predict +60.4% (MAXEnt) (Yeo et al. 2004), +41.6% (Splice Site Prediction by Neural Network-NNSPLICE (Reese et al. 1997) and +8.3% (Splice Site Finder-SSF) (Shapiro and Senapathy, 1987), change at the acceptor site 10bps downstream-ACMG (PP3-not used as PS3 provides a stronger evidence_(Rodriguez-Martin et al. 2005)), the absence of this variant in population databases e.g. GnomAD (Karczewski et al. 2020), and no clinical significance in ClinVar (Landrum et al. 2020) -ACMG PM2). Moreover, the neuropathological examination of the patient with c.823-10G>T *MAPT* variant confirms FTLD and the pathology was very similar to other FTDP-17 patients (Olszewska et al. 2016). Finally, we performed a molecular study on brain tissue from the proband confirming a 4-repeat tauopathy.

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Conclusion:

We expand the phenotype and describe a second family with autosomal dominant 4-repeat FTDP-17 tauopathy associated with the *MAPT* c.823-10G>T intronic variant and, for the first time, present pathological and molecular evidence that this variant is pathogenic. This is the

first description of a family with c.823-10G>T *MAPT* variant accompanied by necropsy and the first description of the MND associated with c.823-10G>T *MAPT* variant.

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Competing interests:

The authors of this manuscript declare that there are no competing interests.

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Figure 1: A Family pedigree depicting autosomal dominant pattern of inheritance, arrow: proband, square: male, circle: female, red: affected with FTDP-17, yellow: affected with late onset dementia, blue: tested for c.823-10G>T variant, green: variant positive, black: variant negative, blue square: necropsy confirmed, diagonal line: deceased. **B** Electeropherogram showing heterozygote change NM_005910.5:c.823-10G>T (III-1) relative to normal sequence (III-5 and III-7) (red arrow), RWS: reverse, FWD: forward.

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Figure 2: Stem-loop structure in the 5splice site of tau exon 10 that regulates alternative splicing, FTDP-17 mutations and -10G>T, intron 9 *MAPT* variant. Variants in green represent previous work of the authors.

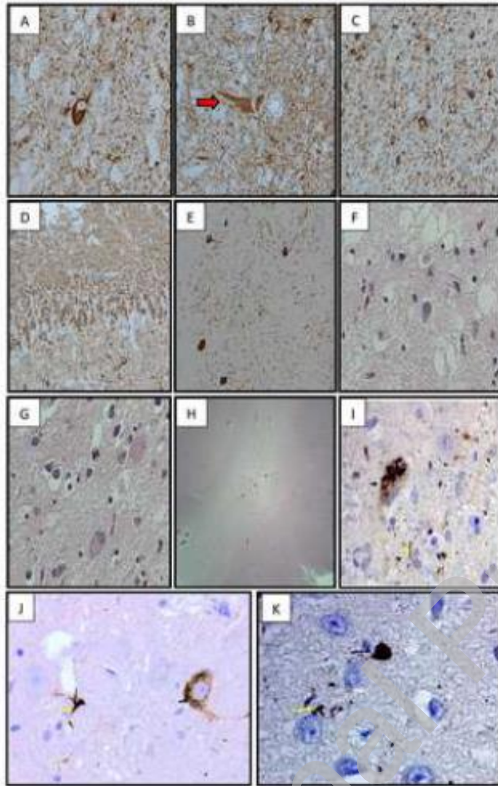

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Figure 3: Post-mortem staining of the proband (III-1) A, B, C, D, E and proband's brother I, J, K, L (monoclonal mouse ant-human PHF-Tau, Clone AT8, Innogen, dilution 1/2000) **A:** Tau-immunopositive neuropil threads and neuronal cytoplasmic aggregates (tangles), x 60, **B:** Tau-immunopositive neuropil threads, diffuse neuronal Tau cytoplasmic immunopositivity (pretangles) (red arrow), x 60, **C:** White matter granular deposits and coiled Tau oligodendrocyte inclusions, x 60 mag, **D:** Circumferential Tau inclusions, dentate fascia neurons of hippocampus, x 20 mag, **E:** Neurofibrillary tangles and neuropil threads, dentate nucleus of cerebellum, x 20 mag, **F** and **G:** H&E stain, cytoplasm distended /

ballooned with basophilic refractile material x 60 mag, (**F:** Neurons in temporal cortex, **G:** Superior frontal cortex), **H:** Substantia nigra with loss of pigmented neurons, gliosis and pigment incontinence, x 2 mag, H&E. Absent Pick bodies **I** 4 R Tau as deposits in neurones and coiled bodies in substantia nigra (yellow arrow) and threads (black arrow), x 40 **J:** 4R Tau in pontine nuclei neurones (black arrow) and as coiled bodies (yellow arrow) x 40, **K:** 4R Tau in parahippocampus gyrus in neurones (black arrow) and as coiled bodies (yellow arrow) x 60.

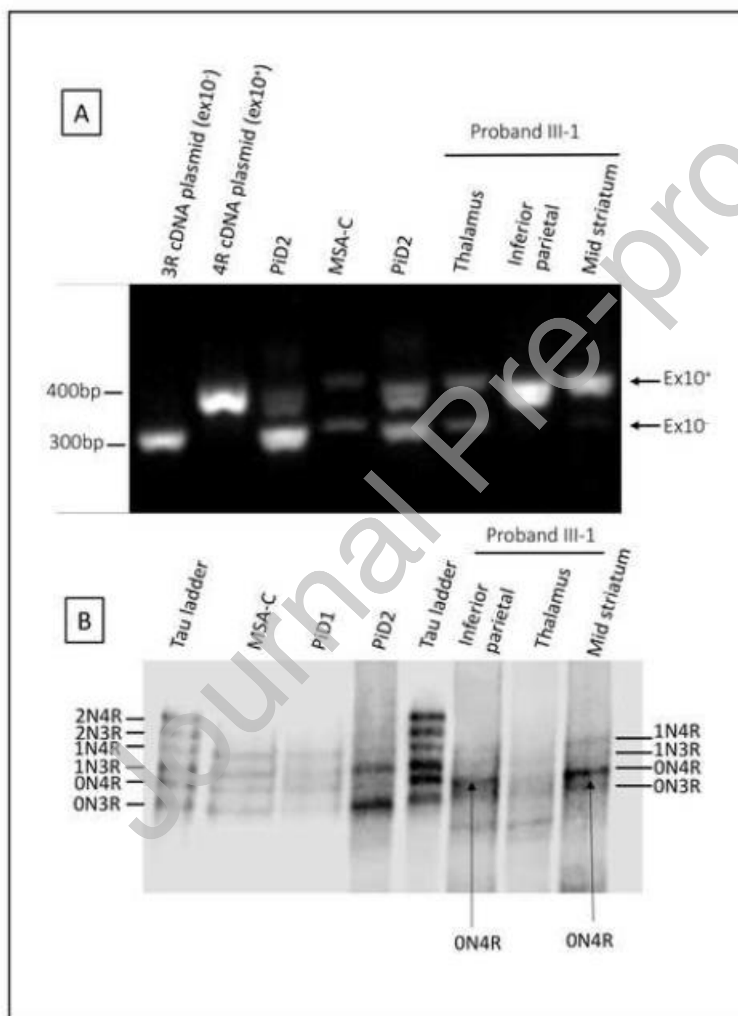

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Figure 4 **A** Proband (III-1): RT-PCR with RNA isolated from 3 brain sections (inferior parietal, mid striatum, thalamus) with primers flanking *MAPT* exon 10. The two PCR products at 397bp and 305bp correspond to 4R-tau- (exon 10^{+}) and 3R-tau (exon 10-)

mRNA, respectively. Note doublet with 4R-tau- (exon 10^{+}) band (397bp) with intermediate band of about 380bp between those for the 3R and 4R-tau mRNA bands (305 bp and 397bp, respectively). Using capillary electrophoresis of fluorescent FAM-labelled PCR product, we confirmed that this is a heteroduplex artefact, as previously described (Grover et al., 1999, Kalbfuss et al., 2001). Two controls (MSA-C and PiD were included in the analysis). The proband samples showed distinctly increased 4R:3R-tau mRNA ratio compared with the controls. **B** Immunoblots of dephosphorylated sarkosyl-insoluble tau extracted from 3 brain regions sections (inferior parietal, mid striatum, thalamus) and compared to control patients with MSA-C (1 sample), Pick's disease (2 samples). The insoluble pathological tau from the proband was almost exclusively 4R-tau.

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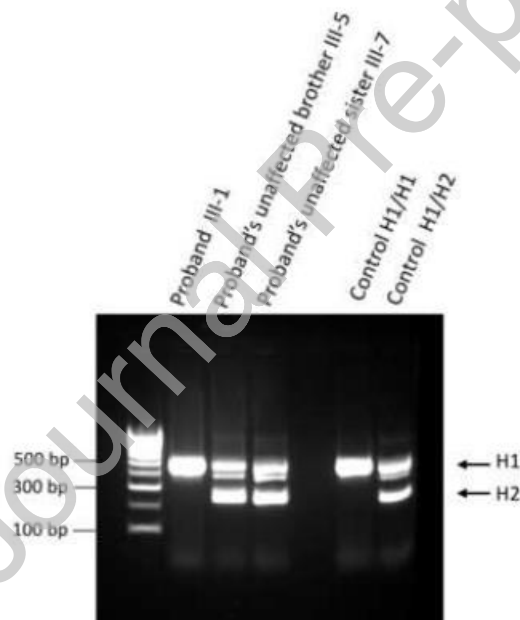

Figure 5 The haplotype analysis for the proband (III-1), and two unaffected siblings (III-5, III-7) compared to control patients (H1/H1 and H1/H2). The intervening band in the H1/H2 heterozygote is a heteroduplex artefact of the two PCR products. The analysis confirmed the H1 background for the proband.

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