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Closing the tau loop: the missing tau mutation

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Frontotemporal lobar degeneration comprises a group of disorders characterized by behavioural, executive, language impairment and sometimes features of parkinsonism and motor neuron disease. In 1994 we described an Irish-American family with frontotemporal dementia linked to chromosome 17 associated with extensive tau pathology. We named this disinhibition-dementia-parkinsonism-amyotrophy complex. We subsequently identified mutations in the *MAPT* gene. Eleven *MAPT* gene splice site stem loop mutations were identified over time except for 5' splice site of exon 10. We recently identified another Irish family with autosomal dominant early amnesia and behavioural change or parkinsonism associated with the 'missing' +15 mutation at the intronic boundary of exon 10. We performed a clinical, neuropsychological and neuroimaging study on the proband and four siblings, including two affected siblings. We sequenced *MAPT* and performed segregation analysis. We looked for a biological effect of the tau variant by performing real-time polymerase chain reaction analysis of RNA extracted from human embryonic kidney cells transfected with exon trapping constructs. We found a c.915+15A>C exon 10/intron 10 stem loop mutation in all affected subjects but not in the unaffected. The c.915+15A>C variant caused a shift in tau splicing pattern to a predominantly exon 10+ pattern presumably resulting in predominant 4 repeat tau and little 3 repeat tau. This strongly suggests that the c.915+15A>C variant is a mutation and that it causes frontotemporal dementia linked to chromosome 17 in this pedigree by shifting tau transcription and translation to +4 repeat tau. Tau (*MAPT*) screening should be considered in families where amnesia or atypical parkinsonism coexists with behavioural disturbance early in the disease process. We describe the final missing stem loop tau mutation predicted 15 years ago. Mutations have now been identified at all predicted sites within the 'stem' when the stem-loop model was first proposed and no mutations have been found within the 'loop' region as expected. Therefore we 'close the tau loop' having 'opened the loop' 21 years ago.

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Abbreviations: FTD = frontotemporal dementia; DDPAC = disinhibition-dementia-parkinsonism-amyotrophy complex; FTDP-17 = frontotemporal dementia with parkinsonism linked to chromosome 17; RBANS = Repeatable Battery for the Assessment of Neuropsychological Status

Introduction

Frontotemporal lobar degeneration comprises a group of disorders characterized by behavioural, executive and language impairment, sometimes with features of parkinsonism or motor neuron disease. Twenty-one years ago we described an Irish-American family with frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) associated with frontotemporal atrophy, superficial cortical spongiform change, neuronal loss, gliosis, destruction of nigra and amygdala and extensive tau pathology (Lynch *et al.*, 1994; Wilhelmsen *et al.*, 1994; Sima *et al.*, 1996). We named this disorder disinhibition-dementia-parkinsonism-amyotrophy-complex (DDPAC). We, and others, subsequently identified mutations in the microtubule-associated protein tau (*MAPT*) gene on chromosome 17q21.31 (Hutton *et al.*, 1998; Spillantini *et al.*, 1998). It is now recognized that 25–50% of frontotemporal lobar degeneration cases are hereditary and 15–20% of these carry mutations in *MAPT* (Onyike *et al.*, 2013), 20–25% carry progranulin (*GRN*) mutations (Baker *et al.*, 2006; Cruts *et al.*, 2006, Borroni *et al.*, 2008) and 3–48% carry *C9orf72* mutations (DeJesus-Hernandez *et al.*, 2011; Renton *et al.*, 2011; Porta *et al.*, 2015). Tau is a natively unfolded microtubule-associated protein concentrated in the axons of maturing and formed neurons and is critical for neuronal function (Wang and Liu, 2008). Hyperphosphorylated tau forms a core component of the intracellular filamentous deposits that characterize a number of neurodegenerative diseases (Rizzu *et al.*, 1999; Wang and Liu, 2008; Spillantini and Goedert, 2013). Tau is involved in promoting neurite outgrowth, organizing axonal microtubules and both dynein and kinesin-dependent axonal transport (Magnani *et al.*, 2007; Wang and Liu, 2008; Morfini *et al.*, 2009). Tau isoform ratios are not conserved across the species. In adult rodents only 4R isoforms are expressed whereas in chickens 3R, 4R and 5R isoforms are present. Six tau isoforms are expressed in the adult human brain. These are produced by the alternative splicing of mRNA of the *MAPT* gene (Goedert *et al.*, 1989). These isoforms differ from each other via the inclusion or exclusion of a 29 or 58 amino acid insert in the amino-terminal half (encoded by exons 2 and 3), and by the inclusion or exclusion of a 31 amino acid sequence that encodes the extra-repeat (encoded by exon 10) in the carboxy-terminal half of the tau gene.

Inclusion of exon 10 (10+) results in 4R tau and exclusion of exon 10 (10−) results in 3R tau. In adults the ratio of 3R:4R is closely regulated at ~1:1 (Goedert and Jakes, 1990; Kar *et al.*, 2011). Tau binds to microtubules using these repeat domains and notably 4R tau binds more efficiently than 3R (Goedert *et al.*, 1990).

MAPT mutations may alter tau protein structure or disrupt the normal 3R:4R tau isoform ratio of tau isoforms (Goedert and Jakes, 2005). The original Irish-American family (DDPAC) (Lynch *et al.*, 1994; Wilhelmsen *et al.*, 1994) carried a +14 C>T exon 10/intron 10 boundary mutation. A cluster of mutations close to this 5'splice site of *MAPT* exon 10 were identified (−2, −1, +3, +12, +13, +14, +16) (Hutton *et al.*, 1998; Spillantini *et al.*, 1998; Grover *et al.*, 1999; Iijima *et al.*, 1999; Stanford *et al.*, 2000; Yasuda *et al.*, 2000). By 2000 structural studies had identified the residues in the stem loop where changes would have caused instability (Varani *et al.*, 2000) and indeed Hutton (2000) predicted that further undiscovered stem-loop mutations would be found. Eleven splice site mutations were subsequently identified over time at each of the sites predicted to destabilize the stem loop, except for the elusive c.915+15A>C mutation (Qian and Liu, 2014).

We have recently identified another Irish family, with branches in the UK, in which the proband showed early amnesia and behavioural change associated with the 'missing' c.915+15A>C mutation at the intronic boundary downstream of exon 10.

Materials and methods

We identified a young adult male with FTDP-17 and recruited a number of his siblings as part of a prospective study looking at the earliest symptoms and biomarkers of frontotemporal lobar degeneration *MAPT* carriers.

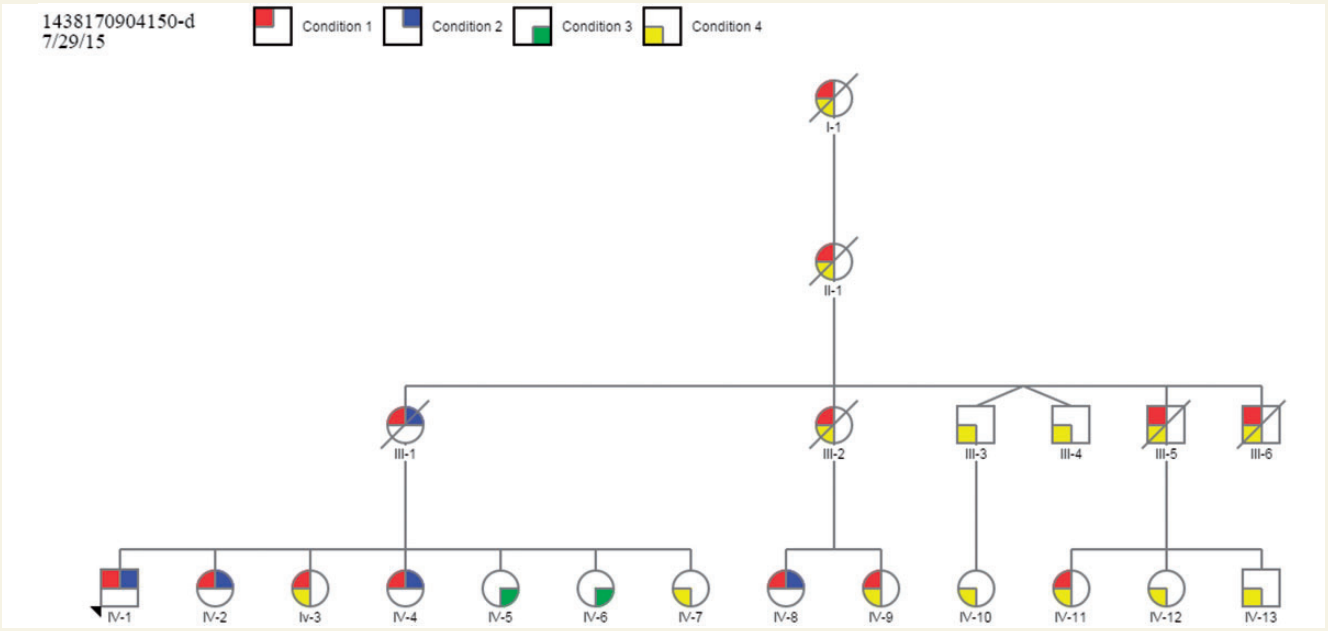


Figure 1 Patient's family history of a young-onset behavioural, amnestic syndrome, suggesting an autosomal dominant pattern of inheritance. proband; male; male twins; affected male; deceased male; female; affected female; deceased female; carrier c.915 + 15A > C; non-carrier; not screened.

We performed a large family study involving five siblings including three affected (Fig. 1). All Irish patients were prospectively assessed in Dublin Neurological Institute at the Mater Misericordiae University Hospital. The study was approved by the hospital Ethics Committee and informed consent was obtained from each participant. Medical records were reviewed and past medical history, family history, social history and medication use were gathered. We performed a full neurological examination on all patients. Brain neuroimaging [MRI, ^{18}F-fluorodeoxyglucose (FDG) PET/CT], EEG, CSF analysis and basic laboratory blood tests were obtained from the proband.

We analysed CSF total tau content, phosphorylated tau and CSF amyloid-β. CSF was collected in sterile polypropylene tubes from the L3/L4 interspace, centrifuged at 2500*g* for 10 min at 4°C and the supernatant aliquoted, and stored at −80°C. Total tau, phospho-tau181 and amyloid-$\beta_{1\text{-}42}$ concentrations were measured using the Innotest® hTau Ag, Innotest® Phospho-tau(181P) (Innogenetics) and MSD Multi-Array® Human Abeta 42 (MSD) ELISA kits (O'Dowd *et al.*, 2013).

MRI brain and basic laboratory tests were also obtained from the two affected siblings. Blood samples for DNA extraction from affected and unaffected siblings were obtained. DNA was also extracted from archived post-mortem brain tissue from the proband's deceased mother. Fluorescent Sanger sequence of exons 1–5, 7, 9–13 of the *MAPT* gene in the index case was analysed using Mutation Surveyor® software developed by SoftGenetics®, LLC. Mutations were named according to HGVS nomenclature and reference sequence NM_005910.5 where +1 is the A of the first coding ATG. Dosage analysis of the *MAPT/GRN* genes was performed using MRC Holland MLPA kit P275-C1.

The genetic variant identified in intron 10 was subsequently tested in other family members for segregation analysis within the pedigree.

To demonstrate a possible biological effect of this new tau variant (c.915 + 15A > C) on *MAPT* transcription we performed real-time PCR analysis on RNA extracted from human embryonic kidney cells (HEK cells) transfected with exon trapping constructs containing mutant and wild-type exon 10 exonic and surrounding intronic sequence (this work was done under Prof. Michael Hutton's supervision at Eli Lilly and Company). The real-time PCR products were separated on an Agilent DNA 1000 chip and quantified using the Agilent 2100 Expert software (Fig. 4).

Results

Family study

The proband (Patient IV–1; Fig. 1) is a 44-year-old right-handed farmer with a 4-year history of a progressive amnestic syndrome, disinhibition, impulsivity, apathy, lack of motivation and *Witzelsucht* (altered sense of humour, a tendency to make puns, tell inappropriate jokes or pointless stories). The profound amnestic syndrome occurred in parallel with the behavioural change. The patient, described as a 'workaholic', could no longer work for a living because of apathy. Collateral history was obtained from his wife as the patient denied any illness.

Past medical history was unremarkable, and he had no history of mood disturbance. Alcohol intake was moderate, and he did not smoke tobacco. His wife described gradual

onset of reduced initiative, becoming increasingly withdrawn with difficulty recalling names. He resorted to list-making for daily tasks.

He had disinhibited, jocular behaviour in public, was less cautious with money, and uninterested in his appearance and hygiene. His mood was labile, fluctuating between avolition and impulsivity, without insight. He played practical jokes; his wife described a 'quirky' sense of humour. On one particular occasion he put on a Halloween mask in a shop and ran around in a 'childlike' way. He became involved in a local lottery, which he avoided in the past.

Reading, counting and spatial orientation skills were intact; he was continent and continued to drive a car. He craved and hoarded sweet foodstuffs. He described no sudden jerks or difficulty walking and family reported he neither had hallucinations nor cognitive fluctuation.

Prosody was flat, but his language was normal. Flat affect improved partially on sertraline. Donepezil (10 mg once daily) was subsequently added, without effect. The patient's family tree (Fig. 1) was consistent with autosomal dominant inheritance of young-onset dementia, with prominent behavioural changes in some family members in four generations. The patient's deceased mother (Patient III–1; Fig. 1) had a behavioural change and memory loss in her early fifties; she developed a 'sweet tooth', a shuffling gait by the age of 54 and died of bronchopneumonia at the age of 57. At autopsy brain weight was 1150 g. Unfortunately brain histopathology was not performed.

The proband's first cousin (Patient IV-8; Fig. 1), developed behavioural symptoms and memory problems by age 45. Short-term memory gradually deteriorated and she forgot names of good friends and had to make a list to shop. There was no language difficulty. She was diagnosed with frontotemporal dementia (FTD). She is currently 51 years old and wanders but lives at home with family support. Her sister (Patient IV-9; Fig. 1) developed behavioural problems by age 43. She began to wander, and was awkward and nervous in company (e.g. wandering off at a wedding to a hotel room without concern that others were worrying about her whereabouts). She developed a 'sweet tooth' with cravings for cream cakes. She was unconcerned and unsafe when crossing a busy road. She was unable to adjust to a change in her commute from a train to a bus and repeatedly was found walking beside the railway tracks. By age 49 she moved to sheltered accommodation because of safety concerns. Her mother (Patient III-2; Fig. 1) was diagnosed at age 50 with 'Alzheimer disease' (brain autopsy was not performed) and she died at age 55 years in a nursing home. The proband's uncle (Patient III-5; Fig. 1) developed memory difficulties in his fifth decade and an 'odd gait'. He was diagnosed with 'Alzheimer disease' in his early fifties and spent his last 4 years of life in a nursing home before dying in his sixth decade. Again no post-mortem was performed.

The uncle's daughter (Patient IV-11; Fig. 1) developed behavioural change in her fifth decade and developed a 'sweet tooth' for cream cakes. She was treated for an obsessive-compulsive disorder and was diagnosed with FTD by age 49 years.

The proband's other uncle (Patient III-6; Fig. 1) developed memory and behavioural problems without a diagnosis and died of a gastric cancer in his eighth decade.

At presentation the proband's neurological examination revealed the presence of primitive reflexes (palmomental, grasp reflexes and glabellar tap), passivity, with occasional jocularity and disinhibition. There was no myoclonus, fasciculations or extrapyramidal signs.

A Montreal Cognitive Assessment of the proband (Patient IV-1; Fig. 1) revealed a score of 25/30 (missing four for delayed recall and one for abstract reasoning). The patient had two neuropsychological assessments performed, 5 months apart. On each occasion he was disinhibited, making numerous sexually inappropriate comments and had difficulty engaging with the assessment process. He had low average levels of performance on tests of vocabulary but impairments were noted on general knowledge testing from the Wechsler Adult Intelligence IV Test (Table 1).

On a list learning task, his immediate word list recall was weak but he showed a satisfactory learning curve over time. However, there was a rapid loss of information from memory with no recall of the list after a brief delay. This remained stable at the second assessment with adequate encoding but poor retention. A similar pattern was seen for prose passage recall with satisfactory immediate recall but little recollection after a delay period. The recognition testing for the list was performed and he was impaired at 12/20 and this pattern was maintained at Time 2. In our view this relates to a retention deficit rather than any frontally mediated retrieval deficit (although poor encoding could adversely affect later retrieval, but recognition should usually improve this too). Confrontational naming was impaired on both occasions but repetition and naming from auditory description were intact. Praxis was intact as was dot counting, fragmented number perception and functional reading with no evidence of dyslexia.

His initial score on the Frontal Assessment Battery at 46 years was 18/18 and 6 months later was 15/18. His overall score in the Haying Sentence Completion Test, a measure of behavioural inhibition, was within the low average range on both occasions. His error pattern revealed difficulty inhibiting lexical responses but these errors were not frankly disinhibited. On the Brixton Spatial Anticipation Test, a measure of mental flexibility and rule following, his initial score fell within the average range, and low average 5 months later. Performance on a phonemic fluency task (F, A, S test) lay within the average range at both testing sessions. Semantic fluency lay within the low average range. Digit Span lay within the average range. Overall his neuropsychological profile revealed deficits in retention of information with confrontational naming deficits and some mild inhibitory deficits on formal testing but all other cognitive skills, especially executive function, fell within normal

Table 1 Neuropsychological test scores of the proband

Test	Raw Score Time 1	Standardized Score Time 1	Raw Score Time 2 (6 months later)	Standardized Score Time 2
RBANS List Learning Immediate recall	22	Scaled Score 6	22	Scaled Score 6
RBANS List Learning Delayed Recall	0	0.04 percentile	1	0.02 percentile
RBANS Recognition	12/20	<0.001 percentile	13/20	<0.0001 percentile
RBANS Story Immediate Recall	16	Scaled Score 9	18	Scaled Score 11
RBANS Story Delayed Recall	3	0.05 percentile	0	<0.0001 percentile
RBANS naming	5/10	0.003 percentile	8	10th percentile
Category Fluency	18	Scaled Score 8	-	-
Digit Span	11	Scaled Score 11	-	-
FAS test	36	9	37	9
Hayling 1: Sensible Completion	8	6 (average)	6	6
Hayling 2: Inhibition time	15	6 (average)	6	6
Hayling 2: Overt Inhibition errors	1	Normal	0	Normal
Hayling B: Somewhat connected errors	9	Impaired	10	Impaired
Hayling Overall	4	Low average	4	Low Average
Brixton Test	15	6 (average)	23	4 (low average)
Frontal Assessment Battery	18/18	Normal	15/18 (0 for inhibitory control)	
WAIS-IV: Vocabulary	22	6	-	-
WAIS-IV: Information	5	4	-	-

FAS = F.A.S: phonemic fluency test; WAIS = Wechsler Adult Intelligence Scale®.
Raw scores are presented for specific subtests as performance was so low. The RBANS is a battery of neuropsychological screening tests where the scaled score average is 7–13. The FAS fluency test also has a scaled score range of 7–13. The Hayling test is a measure of behavioural inhibition and the scaling is 1–10 with 6 representing the average performance. Repetition, praxis and perception within normal limits on each test occasion.

limits except for a slight deterioration in executive tests over a 5-month period. These were in contrast to his marked behavioural disinhibition both socially and during the testing process.

Investigations

MRI brain of the proband at age 45 (Patient IV-1; Fig. 1) showed bilateral mild-to-moderate anterior temporal lobe atrophy, without marked frontal atrophy (Fig. 2A and B). FDG/PET brain at age 46 demonstrated low anterior temporal lobe metabolic activity with normal frontal activity (Fig. 2C). Follow-up FDG/PET at age 49 showed a subtle worsening of low anterior temporal lobe metabolic activity. Laboratory investigations were normal, including full blood count, erythrocyte sedimentation rate, blood film, autoantibody, vasculitis and infectious screen [including Venereal Disease Research Laboratory Test (VDRL) and HIV], urinalysis, anti-neuronal antibodies [voltage-gated potassium channel antibodies (anti-VGKC) and *N*-methyl D-aspartate receptor antibodies (anti-NMDA)]. EEG was within normal limits.

CSF protein content was 51.7 mg/dl (reference range 15–45 mg/dl) and other routine CSF measures were normal. We found that CSF total tau content was 162.1 pg/ml (reference range 65–330), phosphorylated tau at 42.7 pg/ml (reference range 25–82), and CSF amyloid-β at 501 pg/ml (reference range 212–996) supporting a non-amyloid based process. Fluorescent sequencing and dosage analysis of exons 1–5, 7, 9–13 of the *MAPT* gene detected a novel unreported sequence variant, c.915+15A>C, at the exon10/intron10 boundary of *MAPT*.

The familial variant was identified in the patient's affected female maternal first cousin (Patient IV-8; Fig. 1), the proband's two sisters (Patients IV-4 and IV-2; Fig. 1) and in the proband's deceased mother (Patient III-1; Fig. 1) DNA from extracted post-mortem tissue. This tau variant has not been previously reported.

Assessment of siblings

We subsequently tested four of the proband's siblings for the familial *MAPT* variant and assessed them neurologically. Two asymptomatic sisters did not carry the variant, but two other sisters with neurological symptoms were found to carry the familial c.915+15A>C *MAPT* variant. Patient IV-4 (Fig. 1) had cognitive and behavioural change and Patient IV-2 (Fig. 1) had parkinsonism with eyelid opening apraxia.

The proband's older sister (Patient IV-4; Fig. 1) had a history of postnatal depression treated with escitalopram (10 mg). She was slightly disinhibited and was not upset about the positive gene.

Test result

On assessment she had short term memory problems with Montreal Cognitive Assessment Test of 24/30 (missing five for delayed recall and one for naming) compared to an initial Montreal Cognitive Assessment Test of 26/30 (missing four for delayed recall) 18 months earlier. Her

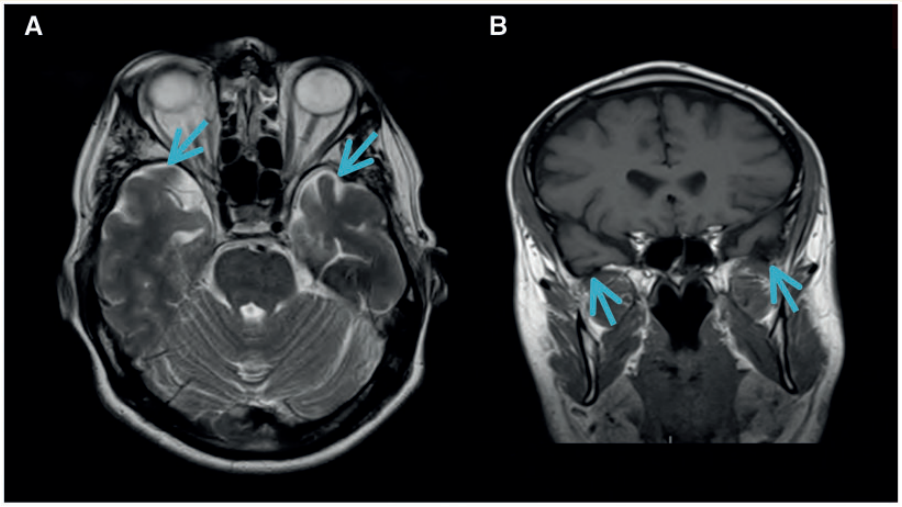


Figure 2 Morphological and functional brain imaging of proband (Patient IV-1) and proband's sister (Patient IV-2). (**A**) MRI brain (axial T_2) showing bilateral mild-to-moderate temporal atrophy indicated by arrow (proband Patient IV-1). (**B**) MRI brain (coronal T_1) showing bilateral mild temporal atrophy more pronounced on the left side, indicated by arrow (proband Patient IV-1). (**C**) FDG-PET scan displaying bilateral reduction in temporal tip activity indicated by arrow with normal frontal and parietal uptake (Proband Patient IV-1; Fig 1). (**D**) Nuclear medicine brain dopaminergic system imaging (^{123}I-FP-CIT DAT scan) of the proband's sister (Patient IV-2; Fig 1). Radiotracer uptake in the striatum was quantified and compared to background activity using automated GE software. Specific ratio values (basal ganglia nucleus compared to background activity) <2.0 may be considered abnormal.

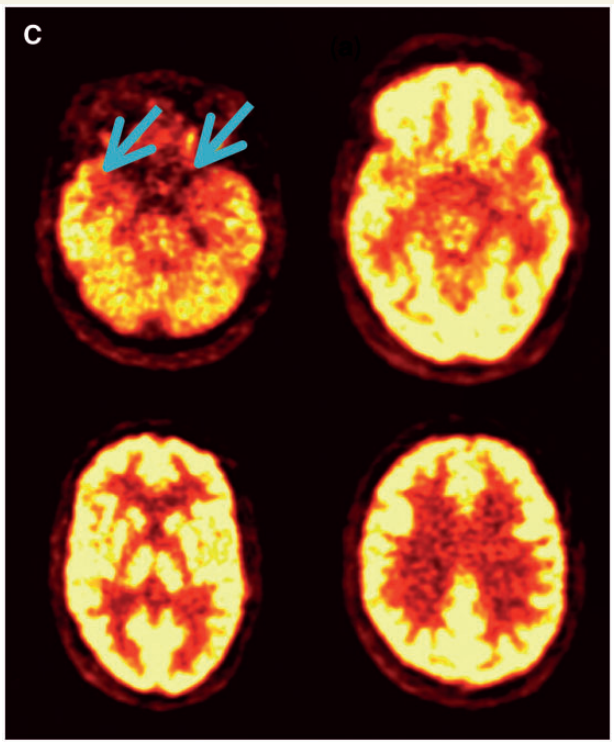
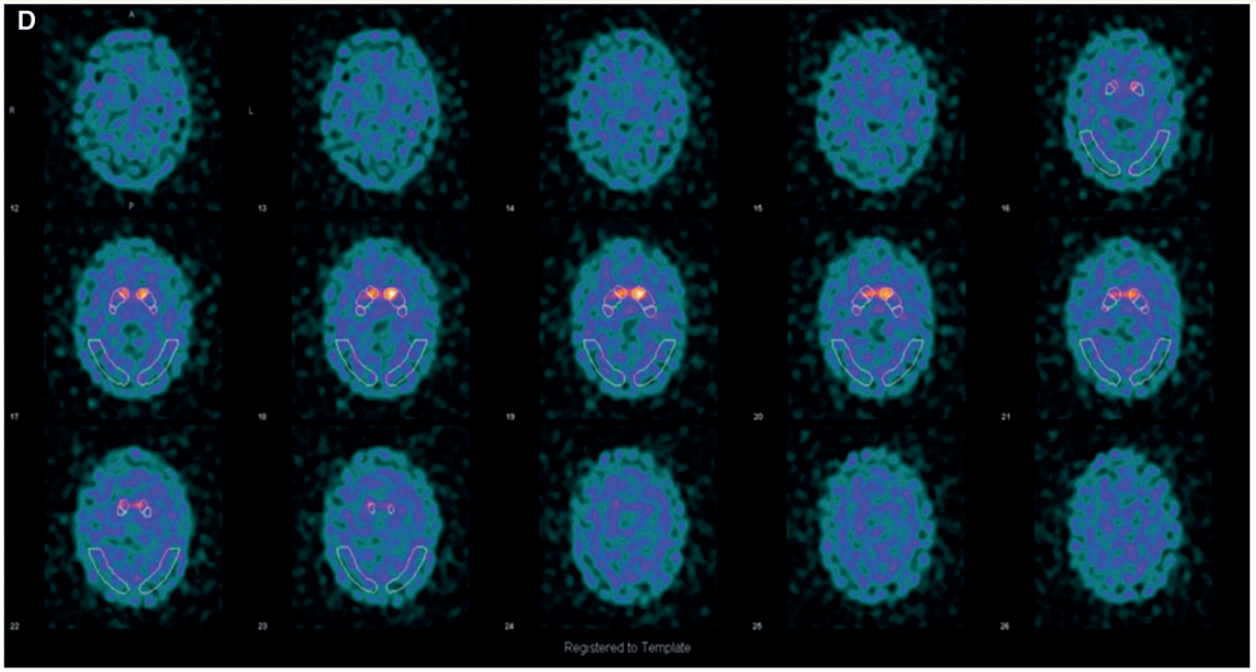


Figure 2 Continued.

Frontal Assessment Battery was at 18/18. Brain MRI was normal. The proband's younger sister (Patient IV-2; Fig. 1) also carrier of the c.915+15A>C *MAPT* familial variant and had a 1-year history of difficulty opening jars, left-sided clumsiness, difficulty opening her mouth, drooling of saliva, slowness, and 6 months of difficulty opening her eyes (eyelid opening apraxia). She had no hallucinations and no language problems. She scored 30/30 on Montreal Cognitive Assessment Test (initial score 18 months prior was 28/30). Her Frontal Assessment Battery was at 18/18. She takes citalopram (20 mg) for depression and anxiety associated with the gene test result. Neurological examination showed a masked facies, eyelid opening apraxia, brisk jaw jerk, brisk reflexes, spastic catch in the left wrist on pronation/supination and in lower limbs, clonus at the left ankle. She had left-sided bradykinesia and side-to-side neck movements were slow. MRI of the brain and cervical spine showed mild involutional change involving temporal lobes bilaterally. Her nuclear medicine brain dopaminergic system imaging (^{123}I-FP-CIT DAT) scan showed a moderate-to-severe reduction in tracer uptake in the striatum bilaterally (Fig. 2C and D). Changes were more prominent on the right side (Fig. 2C). Basic blood test results were normal.

The eldest sister (Patient IV-3; Fig. 1), who was 53 years old and a health-care worker, had behavioural and memory problems but was not formally assessed or genetically tested.

Real time PCR analysis of the products from HEK cells translated from exon trapping constructs showed a shift in the tau splicing pattern resulting in predominantly exon 10+ (246 bp product) pattern as compared to a predominantly exon 10− (153-bp product) pattern in cells transfected with the wild-type construct (Fig. 4A and B). This change is predicted to result in predominant 4 repeat tau (4R) (exon 10+) and little 3 repeat tau (3R) (exon 10−) being generated from the mutant *Tau* allele.



Results by region [Female 39 years old, OSEM (non-corrected)]

	Striatum Right	Striatum Left	Putamen Right	Putamen Left	Caudate Right	Caudate Left	Put/Caud Rate Right	Put/Caud Rate Left
Patient uptake	0.63	0.75	0.45	0.49	0.91	1.23	0.76	0.67

Figure 2 Continued.

Discussion

Seventeen years ago we described multiple *MAPT* mutations associated with FTDP-17 including an exon 10/intron 10 boundary (C>T+14; G>A+3) stem loop mutation associated with FTDP-17, including the DDPAC family (Hutton *et al.*, 1998; Spillantini *et al.*, 1998). Thus we 'opened the *Tau* loop'. Importantly, the identification of mutations in *MAPT* as the cause of FTDP-17 demonstrated that tau dysfunction alone is sufficient to cause neurodegeneration. Tau dysfunction has a central role in FTDP-17 and in other conditions including Alzheimer's disease, progressive supranuclear palsy, Pick disease, corticobasal syndrome and other tauopathies (Murray *et al.*, 2005). The intronic region bordering *MAPT* exon 10 forms a stem loop structure that partially controls the inclusion of exon 10 (leading to expression of 4R tau) or exclusion of exon 10 (leading to expression of 3R tau). Multiple mutations within the stem loop were found but one remained elusive (Hutton, 2000).

We now report the 'missing' c.915+15A>C mutation in a different Irish family, thus 'closing the loop' (Hutton, 2000) (Fig. 3). We confirm that the c.915+15A>C stem loop mutation alters *MAPT* splicing in HEK cells transfected with exon trapping constructs. This generates a shift from the normal 3R:4R pattern of tau isoforms to a predominance of 4R by exon 10+ inclusion as predicted by the reduced stability of the mutant stem-loop structure and similar to the effects observed previously with other stem loop mutations (e.g. −1 U>C) (Fig. 4). This strongly suggests that the c.915 +15 A>C variant is a pathogenic mutation and that it causes FTDP-17 in this pedigree by shifting tau transcription to overproduce 4R tau similar to other FTDP-17 mutations (Hutton *et al.*, 1998; Spillantini *et al.*, 1998).

Most *MAPT* pathogenic mutations are located in exons 9–13 encoding the repeat domains and their adjacent introns. Exonic mutations are missense, silent or deletion in nature. The majority of these mutations reduce the ability of tau to interact with microtubules (Hasegawa *et al.*, 1998) and increase the tendency for tau to assemble into filaments (Nacharaju *et al.*, 1999). In contrast, mutations within exon 10 and the surrounding intronic sequences that alter splicing increase the splicing-in of exon 10 resulting in increased 4R tau. Intronic mutations found primarily at the 5' splice site of the intron following exon 10, are located in the stem loop region and alter the correct functioning of this stem loop (Grover *et al.*, 1999; Varani *et al.*, 2000). The presence of the stem loop structure normally restricts

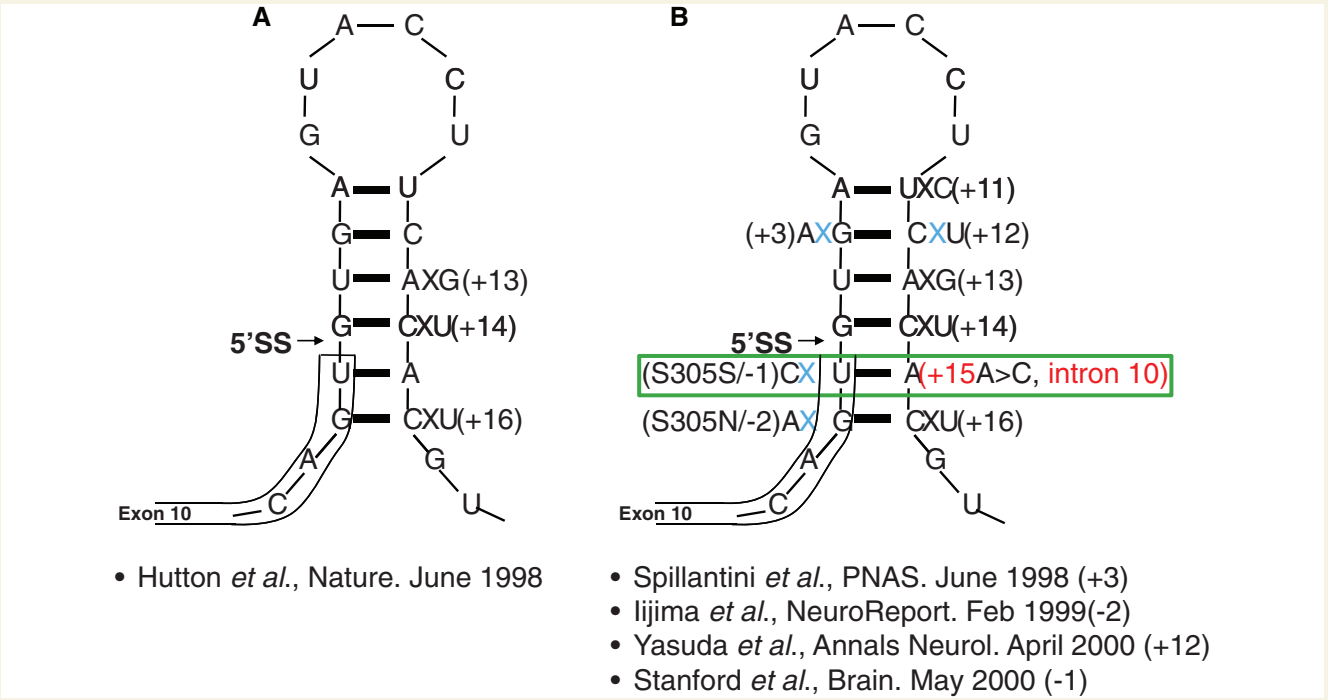


Figure 3 Stem loop structure at the exon 10/intron 10 boundary with the c.915+15A > C change highlighted. A = adenine; G = guanine; C = cytosine; U = uracil; 5'SS = 5 primer splicing site; +3 = familial multiple system tauopathy with presenile dementia (Spillantini *et al.*, 1998); +11 = (Miyamoto *et al.*, 2001; Kowalska *et al.*, 2002); +12 = FTD-Kumamoto (Yasuda *et al.*, 2000); +13 = (Pickering-Brown *et al.*, 2002); +14 = DDPAC (Lynch *et al.*, 1994); +16 = (Lantos *et al.*, 2002). (**A**) The initial identification of stem loop mutations at exon 10/intron 10 boundary (Hutton *et al.*, 1998) +14 is the original Irish-American DDPAC family. (**B**) The stem loop mutations identified, including −2, −1, +3 +11, +12, +13, +14, and +16. The c.915+15A > C mutation was not identified at the time but was predicted to exist (Hutton, 2000).

access of the 5' splice site to the spliceosome, thereby controlling the rate of splicing-in of exon 10. Intronic mutations in the stem region destabilize the stem loop structure thus promoting splicing-in of exon 10 with increased production of the 4R tau isoforms as a consequence (Kar *et al.*, 2011). Mouse models of FTDP-17 including the *MAPT* N279K exon 10 splicing mutation and c.915+16 C>T exon 10/intron 10 stem loop mutations develop neurodegeneration as a result of aberrant splicing and recapitulate many of the disease hallmarks seen in patients with N279K or +16 mutations (Dawson *et al.*, 2007; Umeda *et al.*, 2013).

The precise mechanism by which the shift in exon 10 splicing leads to neurodegeneration is unclear but 4R tau aggregates more readily than 3R tau; the most parsimonious explanation is that the splicing mutations, like the exonic mutations that affect protein structure, cause an increase in tau aggregation (Umeda *et al.*, 2013). Given that patients with these mutations appear normal into their fourth and fifth decades, there is little to suggest that this shift in splicing ratio alone has a major effect on microtubule function.

In this unique FTDP-17 family, the proband and other family members had a simultaneous behavioural syndrome typical for behavioural variant FTD and episodic memory deficits leading us to speculate whether the proband had atypical familial Alzheimer's disease (Rippon *et al.*, 2003). Clinically no parkinsonism or amyotrophy was evident in the proband. However, his younger sister (Patient IV-2; Fig. 1) subsequently presented with parkinsonism with eyelid opening apraxia, without cognition or behavioural change.

On imaging, the proband demonstrated bilateral anterior temporal lobe atrophy and hypometabolism on FTD/PET imaging. The clinical presentation of this family is consistent with the phenotype observed with other *MAPT* mutations which commonly present with symptoms of behavioural variant FTD and bilateral temporal atrophy (Rohrer and Warren, 2011; Whitwell *et al.*, 2012). Parkinsonism may be present in patients with *MAPT* mutations and can rarely be the sole presenting feature. Episodic memory deficits, as observed in the current family, can be seen in patients with *MAPT* mutations, usually in conjunction with symptoms of behavioural variant FTD (Rohrer and Warren, 2011).

Early amnesia was an exclusion criterion in the 1998 clinical criteria for frontotemporal lobar degeneration (Neary *et al.*, 1998), but is permitted within the more recently proposed diagnostic criteria (the International BvFTD Criteria Consortium) (Rascovsky *et al.*, 2011). It is thought that the newer criteria are more sensitive (Costa *et al.*, 2013). Tau screening should therefore be considered as part of the diagnostic work-up in families where amnesia or atypical parkinsonism co-exists with behavioural disturbance early in the disease process. The detection of the novel c.915+15A>C mutation 15 years after its prediction highlights the value of pursuing a complete family history with segregation analysis to guide appropriate molecular genetic testing. With the discovery of this

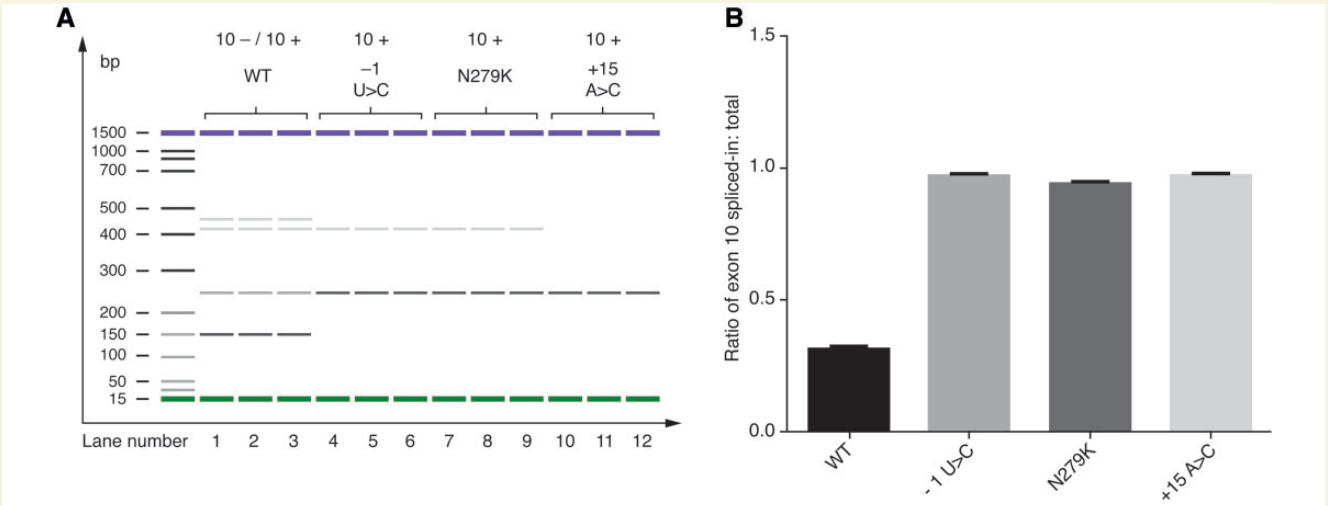


Figure 4 +15-splice-site mutations increase incorporation of tau exon 10 into artificial mRNAs. (**A**) The effect of the c.915 + 15A > C mutation: a clear increase in exon 10 + RNA (10 + /10 − RNA ratio) induced by the c.915 + 15A > C mutation. The c.915 + 15A > C mutation (lanes 10–12) causes the tau splicing pattern to shift from the predominantly exon 10 − (153bp product) pattern seen with the wild-type (WT) stem-loop (lanes 1–3) to predominantly exon 10 + (246 bp product). A second stem-loop destabilizing mutation, −1 U > C (lanes 4–6) and the exonic N279K mutation (lanes 7–9) both caused a shift in splicing pattern to one of predominantly exon 10 +. The bands between 400–500 based pairs represent cryptic splice products linked to the use of an alternative 5'splice site in the construct (the alternative site is sometimes used when the main 5'site is blocked by the stem loop). When the stem loop is disrupted by a mutation the levels of these cryptic splice products are also reduced. Therefore these 400–500 bp products are not seen in the + 15 mutation nor in the N279K mutation nor in the −1U > C mutation (Grover *et al.*, 1999). Real-time PCR products from HEK cells transfected with exon trapping constructs were separated on an Agilent DNA 1000 chip and quantified using the Agilent 2100 Expert software. Bp = base pair; WT = wild-type; U = uracil; C = cytosine; A = adenine; N279K = exon 10 mutation associated with pallido-ponto-nigral degeneration. (**B**) The ratios of tau exon 10 + to total exon 10 (±SD) are shown indicating the predominance of exon 10 + in the splice site mutations −1 U > C and + 15A > C and the exonic mutation N279K.

c.915 + 15A > C *MAPT* pathogenic mutation all mutations have now been identified at all the predicted sites within the 'stem' when the stem-loop model was first proposed and no mutations have been found within the 'loop' region as expected. Moreover no mutations have been found in the intronic sequence beyond the predicted stem-loop structure (+ 17 onwards) that have been shown to be disease-causing. Thus having 'opened the loop' 21 years ago, we now 'close the loop'.

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Supplementary material

Supplementary material is available at *Brain* online.

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