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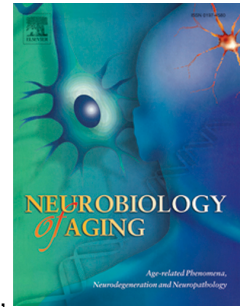
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**Two pathologically confirmed cases of novel mutations in the *MAPT* gene causing frontotemporal dementia**

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**Abstract**

*MAPT* mutations were the first discovered genetic cause of frontotemporal dementia (FTD) in 1998. Since that time, over 60 *MAPT* mutations have been identified, usually causing behavioural variant FTD (bvFTD) and/or parkinsonism clinically. We describe two novel *MAPT* mutations, D252V and G389\_I392del, each presenting in a patient with bvFTD and associated language and cognitive deficits. Neuroimaging revealed asymmetrical left greater than right temporal lobe atrophy in the first case, and bifrontal atrophy in the second case. Disease duration was 8 years and 5 years respectively. Post mortem examination in both patients revealed a 3-repeat predominant tauopathy, similar in appearance to Pick's disease. These two mutations add to the literature on genetic FTD, both presenting with similar clinical and imaging features to previously described cases, and pathologically showing a primary tauopathy similar to a number of other *MAPT* mutations.

## Background

Frontotemporal dementia (FTD) is a clinically and pathologically heterogeneous group of neurodegenerative disorders (Woollacott 2016). Around one third of FTD is familial, with the first gene identified as having causative mutations being microtubule-associated protein tau (*MAPT*) in 1998 (Hutton et al, 1998, Poorkaj et al, 1998, Spillantini et al, 1998). Since that time over 60 *MAPT* mutations have been discovered, with the majority presenting with clinical features of behavioural change and/or parkinsonism (Greaves et al, 2019). Other symptoms include semantic impairment (although it is rare for language problems to be the presenting feature) and, unlike most other forms of FTD, episodic memory difficulties (Tolboom et al, 2010; Liang et al, 2014). Age of symptom onset is variable (although is commonly in the 40's or 50's), as is disease duration, which can vary from between a few years and over twenty (Ygland et al, 2018). Pathologically, there is also heterogeneity: often both 3-repeat (3R) and 4-repeat (4R) isoforms of tau are found in the neuronal and glial inclusions at post mortem but for some mutations 3R-tau inclusions are predominant whilst others have predominantly 4R-tau (Ghetti et al, 2015). In this case series we report two individuals who presented with FTD and were found to have novel pathogenic *MAPT* mutations with subsequent pathological confirmation of a primary tauopathy.

## Methods

### *Clinical*

Both patients attended the Specialist Cognitive Disorders Clinic at the National Hospital for Neurology and Neurosurgery where they underwent a standard clinical and neuropsychological assessment. Magnetic resonance imaging was performed on a 3T Siemens Trio scanner.

### *Genetics*

Blood was taken for genetic testing in both patients with informed consent. Sequencing of exons 1 and 9-13 of the *MAPT* gene was performed initially. Subsequently both samples underwent exome sequencing. Exome enrichment was performed using TruSeq Exome Capture kit (Illumina, San Diego, CA, USA). Sequencing was performed on a HiSeq 2000 (Illumina). Reads were aligned to GRCh37/hg19 using BWA, variants called according to GATK best practice guidelines and annotated with ANNOVAR (Wang et al. 2010). In silico pathogenicity predictions of non-synonymous variants were done with the Combined Annotation Dependent Depletion (CADD) (Kircher et al. 2014). Variants were filtered against population databases (1000Genomes, ESP and gnomAD) and assessed based on variant type (missense, nonsense, splice site, frameshift, non-frameshift) and predicted pathogenicity.

### *Neuropathology*

Both patients consented to brain donation at the Queen Square Brain Bank for Neurological Disorders (QSBB). After death the brains were processed according to QSBB protocol, which involves freezing of the right brain hemisphere, and fixation in 10% buffered formalin with tissue sampling and processing for paraffin histology of the left hemisphere. All tissues are stored at QSBB under a licence from the Human Tissue Authority. Sections of 7  $\mu$ m thickness were cut from formalin-fixed paraffin-embedded tissue blocks from multiple brain regions, mounted on glass slides and stained with haematoxylin and eosin (H&E). Representative sections were examined with immunohistochemical stains with the following antibodies: phospho-tau (AT8 MN1020, 1:600; Thermo), A $\beta$  (6F3D, 1:100; Dako),

$\alpha$ -synuclein (ab80627, 1:1500; Abcam), TDP-43 (2E2-D3, 1:5000; Abnova), 4R tau (1E1/A6, 1:4000; Millipore) and 3R tau (8E6/C11, 1:800; Millipore). All immunostainings were carried out on an automated immunostaining machine (A.Menarini Diagnostics) following manufacturer's guidelines using 3,3'-diaminobenzidine as chromogen and appropriate positive and negative controls. Ethical approval for the study was obtained from the Local Research Ethics Committee of the National Hospital for Neurology and Neurosurgery.

## Results

### Case 1

#### *Clinical history*

A 46-year-old right-handed gentleman presented to his local neurologist with a one year history of behavioural change and impairment of language and cognition. He had become more socially withdrawn and apathetic with poor self-care. He had also become more obsessive with a preference for routines. Over the same time period he had had difficulty with understanding the meaning of words and had developed difficulties with recognising both faces and familiar places, the latter impairment leading to him getting lost at times. He was otherwise fit and well without any other medical problems.

The presentation led to a referral to our clinic where he underwent a detailed cognitive assessment. His speech was noted to be fluent but empty of content with repeated use of stock phrases. MMSE was 22/30. He was anomic with poor single word comprehension and a surface dyslexia.

Neuropsychometric testing revealed a verbal IQ of 73 and performance IQ of 125. He scored below the 5<sup>th</sup> percentile on the Oldfield Naming Test. He named only 2 animal and 7 F words on testing verbal fluency. Posterior cortical function was intact, scoring full marks on the Visual Object Space and Perception battery Incomplete Letters and Cube Analysis subtests. Executive function testing revealed performance within the normal range on the Brixton Spatial Anticipation task (75-90<sup>th</sup> percentile) and the Trail Making Test Part B (25-50<sup>th</sup> percentile).

There was a strong family history of dementia with his mother developing 'dementia' in her 70's, and all but one of her seven siblings also developing 'dementia' in their 60's or 70's. Little was known about the phenotype of his relatives.

MR imaging (Fig 1A) showed bilateral anterior temporal lobe atrophy, particularly affecting the medial temporal lobes including the amygdala and anterior hippocampus, more marked on the left than the right. There was less marked frontal lobe involvement, but particularly involving the left orbitofrontal cortex.

A diagnosis of FTD with both behavioural symptoms and prominent semantic impairment was made at this time.

He was reviewed twice over the next year. Worsening behaviour was noted with hoarding, development of a sweet tooth and inappropriate trusting behaviour. Comprehension had deteriorated with difficulties communicating effectively by the last appointment. He was not

seen again in the clinic, requiring full time care from this point. He died five years later at the age of 54.

### *Genetics*

The patient was found to carry a novel missense variant in exon 9 of the *MAPT* gene: NM\_001123066.3: c.1760A>T: p.Asp587Val. In some nomenclatures of the *MAPT* gene this is known as p.D252V. He subsequently underwent exome sequencing and no other mutations were found in any of the genes known to cause frontotemporal dementia apart from a variant of uncertain significance in *DCTN1* (NM\_004082.4: c.2264dupG: p.Ser755fs). Using CADD to predict the deleteriousness of the *MAPT* variant we obtained a normalized PHRED score of 21.5, indicating this variant is predicted to be in the top 1% of the most deleterious variants in the human genome.

### *Neuropathology*

Macroscopically, with a whole brain weight of approximately 830g, there was global atrophy with emphasis in the temporal and frontal lobes, with widespread cortical thinning, reduction in white matter bulk and severe dilation of the lateral and 3<sup>rd</sup> ventricles (Figure 2, A1-A3). The caudate nucleus showed severe atrophy and the globus pallidus was darkly discoloured whilst the putamen was only mildly reduced in size (Figure 2, A2). The thalamus showed a reduction in size (Figure 2, A3), but the subthalamic nucleus appeared normal. There was very severe atrophy of the hippocampus and the amygdala was not discernible due to the extremely severe atrophy (Figure 2, A3). The size of the brain stem was reduced and in the midbrain there was particularly prominent atrophy of the fronto-



pontine tracts and pallor of the substantia nigra (Figure 2, A4). The locus coeruleus was well pigmented. The cerebellum showed no macroscopic abnormality.

Microscopically, there was severe pan-cortical atrophy, with spongiosis, marked chronic gliosis and severe neuronal loss in the frontal and temporal lobes, including superior temporal gyrus (Figure 2 A7 and A11). In the parietal lobe (inferior parietal lobule more than superior parietal lobule) there was prominent pan-cortical atrophy. Tau immunohistochemistry revealed a meshwork of neuropil threads, pre-tangles, globular inclusions (Pick bodies), and neurofibrillary tangles as well as widespread pan-cortical astrocytic tau pathology in the grey matter of frontal, temporal and parietal lobes (Figure 2 A8 and A12) with a fine meshwork of threads and occasional coiled bodies in the white matter. The majority of neuronal cytoplasmic and thread tau pathology was confirmed to be 3R-tau, whilst a proportion of the astrocytic tau pathology showed labelling with antibodies against 4R-tau (Figure 2, A9-A10). Microscopic examination confirmed the macroscopically evident very severe atrophy of the hippocampal subregions and the amygdala atrophy with particularly widespread atrophy of the dentate gyrus. Across the medial temporal lobe there were phospho-tau (AT8) and 3R-tau positive neuropil threads, Pick bodies, neurofibrillary tangles and pre-tangles (Figure 2, A13-A14). In the severely atrophic caudate, the less atrophic globus pallidus, putamen and thalamus, there was a dense phospho-tau and 3R-tau immunoreactive neuropil thread and globular and globose neuronal cytoplasmic tau pathology. Severe neuronal loss with free pigment deposition and prominent chronic gliosis was seen in the substantia nigra accompanied by dense phospho-tau immunoreactive thread and tangle pathology across the midbrain. The corticospinal tracts across the brain stem showed severe atrophy. Whilst the overall cerebellar cytoarchitecture was preserved, scanty

tau pathology was seen in the cerebellar white matter, with more frequent threads and pre-tangles evident across the dentate nucleus. Histological examination revealed no evidence of A $\beta$ ,  $\alpha$ -synuclein or TDP-43 pathology and assessment of Braak & Braak neurofibrillary tangle stage was not feasible due to the presence of the primary tauopathy.

## Case 2

### *Clinical history*

A 49-year-old woman presented to our clinic with a 2-year history of behavioural change, language impairment and poor planning and decision making. She had become more apathetic with a decline in self-care. There was increasing word-finding difficulty with problems at home suggestive of executive dysfunction e.g. difficulties following instructions and difficulty operating the cooker.

The patient's mother died in middle age without a diagnosis of dementia, though aggressive behavior was reported. However, her mother's mother developed dementia with behavioural change in her early 50's, dying at the age of 70.

On examination, speech was fluent but with clear word-finding difficulties. MMSE was 16/30. Executive function was impaired with poor cognitive estimates and concrete interpretation of proverbs.

Neuropsychometric testing revealed a verbal IQ of 54 and a performance IQ of 60. She named only 3 animal and 2 S words on testing verbal fluency. She was globally impaired with problems in episodic memory (<5<sup>th</sup> percentile on the Topographical Recognition

Memory Test), naming (<5<sup>th</sup> percentile on the Oldfield Naming Test), visuospatial skills (<5<sup>th</sup> percentile on the Position Discrimination task of the Visual Object Space and Perception battery), and executive function (failure on the Weigl Sorting Task). Visuo-perceptual skills however were relatively intact (normal performance on the Incomplete Letters task of the Visual Object Space and Perception battery).

MR imaging (Figure 1B) showed bilateral, relatively symmetrical, frontal (mainly dorsolateral prefrontal cortex) more than temporal lobe atrophy.

A diagnosis of FTD with both behavioural symptoms and severe cognitive difficulties was made at this time.

She was reviewed one year later when her speech had deteriorated with severe word-finding difficulties and minimal speech. Behaviour had deteriorated with increasing emotional lability.

She was then seen a further two years later by which time she required full time care and was doubly incontinent with impaired mobility. She was not seen again in the clinic and died 2 years later at the age of 54.

### *Genetics*

The patient was found to carry a novel variant in exon 13 of the *MAPT* gene: NM\_001123066.3: c.2171\_2182del: p.Gly724\_Ile727del. In some nomenclatures of the *MAPT* gene this is known as p.G389\_I392del. This is a heterozygous deletion of twelve nucleotides

leading to a deletion of 4 amino acids. Conservation assessment of the deleted region using phastCons produced scores between 0.901 and 1 reflecting a high probability of the deleted nucleotides belonging to a conserved element of the genome. Using CADD to predict the deleteriousness of this variant we obtained a normalized PHRED score of 21.5, indicating this variant is predicted to be in the top 1% of the most deleterious variants in the human genome. Of note, two point mutations, both reported to be pathogenic, have been previously described in a residue included in this deletion: NM\_001123066.3:c.2170G>A:p.Gly724Arg (or p.G387R: Pickering-Brown et al, 2000; Bermingham et al, 2008) and NM\_001123066.3:c.2170G>C: p.Gly724Arg (or p.G387R: Murrell et al, 1999; Ghetti et al, 2000).

### *Neuropathology*

Macroscopically, with a whole brain weight of approximately 970g, there was severe frontal and anterior and medial temporal lobe atrophy with a thin and discoloured cortical ribbon (Figure 2, B1, B2, B3). There was also prominent atrophy of the inferior parietal lobule and enlargement of the frontal horn of the lateral ventricle, and to a lesser extent, the 3<sup>rd</sup> ventricle. All deep brain nuclei were mildly reduced in size (Figure 2, B2, B3). Prominent atrophy of the amygdala and hippocampus was observed, but the superior temporal and Heschl's gyri were preserved in bulk (Figure 2, B3). The bulk of the brainstem was reduced and the fronto-pontine tracts in the midbrain and corticospinal tracts in the pons showed marked atrophy. The substantia nigra showed pallor rostrally at the level of the red nucleus (Figure 2, B4), and also locus coeruleus was pale. The cerebellum was unremarkable.

Microscopically, there was very severe pan-cortical atrophy of the anterior frontal lobe, middle temporal (but not superior temporal or Heschl's) gyri (Figure 2, B7 and B11) and inferior parietal lobule. Immunostaining for phospho-tau (AT8) confirmed a primary tauopathy with a dense meshwork of pan-cortical neuropil threads and frequent globular 3R-tau immunoreactive neuronal inclusions (Pick bodies) across the frontal, temporal and parietal lobes. 3R-tau immunostaining also highlighted occasional astrocytic inclusions, whilst 4R-tau positive inclusions were absent (Figure 2, B8, B9, B10 and B12). Tau pathology in the form of scattered threads and occasional coiled bodies was also seen in the subcortical white matter of frontal, temporal and parietal lobes. The atrophic hippocampus and amygdala contained dense tau positive thread and tangle pathology with frequent 3R-tau positive threads and Pick bodies and globose tangles in the residual neurones (Figure 2, B13 and B14). Dense neuropil thread and frequent neuronal tangle and Pick body pathology was seen throughout the caudate, Meynert nucleus, internal part of the globus pallidus and medial aspect of the putamen, with comparably less tau pathology in the external part of the globus pallidus, lateral aspect of the putamen and thalamus. The mildly atrophic substantia nigra, locus coeruleus and pontine base contained widespread tau pathology in the form of neuropil threads and tangles. In the cerebellum there were moderate numbers of pre-tangles and neuropil threads in the dentate nucleus, with no apparent tau pathology in the cerebellar cortex or white matter. Histological examination also showed A $\beta$  parenchymal pathology corresponding to Thal phase 2 and CERAD score 0. Assessment of the neurofibrillary tangle tau pathology and corresponding Braak and Braak stage was not possible due to the severity of primary tauopathy. There was no evidence of additional  $\alpha$ -synuclein or TDP-43 pathology.

## Discussion

We have described two novel *MAPT* mutations presenting as FTD with both behavioural symptoms and prominent semantic impairment. Although segregation data is not available for either mutation, both mutations are predicted to be pathogenic, and importantly both are associated with a primary tauopathy at post-mortem.

These patients presented with FTD with behavioural symptoms and cognitive deficits including semantic impairment; previous reports of the phenotype of *MAPT* mutations are consistent with this e.g. in a large series from Manchester many patients had associated semantic impairment (evidenced by anomia, semantic naming errors and impaired word and/or object comprehension) in conjunction with behavioural change (Snowden et al, 2015). Both of the patients had features of episodic memory impairment; although unusual for FTD, amnesic syndromes are seen in carriers of *MAPT* mutations e.g. R406W (Tolboom et al, 2010), Q351R (Liang et al, 2014) and duplications (Rovelet-Lecrux et al, 2010). The magnitude of episodic memory impairment can mirror that of Alzheimer's Disease (AD), and therefore it is not uncommon for clinical AD to be diagnosed initially in some patients with *MAPT* mutations. Many patients will develop parkinsonian features, including features consistent with a CBS phenotype (or less commonly a PSP phenotype) (Benussi et al, 2015) although this was not seen in our two patients.

Age at onset was 45 in case 1, with a disease duration of 9 years, whilst age at onset was 47 in case 2, with a disease duration of 7 years, both of which are consistent with previous reports. In general, patients with an underlying pathogenic *MAPT* mutation have a mean age at onset in the mid-50s (Snowden et al, 2015), though onset can be anywhere between

the third to the ninth decade. Some mutations tend to cause a more rapidly progressing disease (e.g. P301L) and others a more slowly progressive phenotype (e.g. R406W), although with many cases, like the ones described here, disease duration lies somewhere in between these two extremes.

Although the two mutations described here are novel, mutations close to these sites have previously been shown to be pathogenic. Close to the D252V mutation in exon 9 (in case 1), the K257T mutation has been reported to present in the fifth decade with an amnesic and then behavioural syndrome (Rizzini et al. 2000), in keeping with our patient. Similar to the G389\_I392del mutation (in case 2), mutations in the G389R are associated with disease onset between 17-53 years and typically present with a behavioural syndrome with or without parkinsonism (Tacik et al. 2017, Chaunu et al. 2013, Bermingham et al. 2008, Murrell et al. 1999, Rossi et al. 2008, Pickering-Brown et al. 2000).

Typical patterns of atrophy have been identified in large cohort studies of genetic FTD, which suggest an association with relatively symmetrical anterior and medial temporal lobe involvement (Cash et al, 2018, Fumagalli et al, 2018) though asymmetrical bitemporal involvement has also been described (Rohrer et al, 2010), similar to the first case. However, some cases have more frontal involvement such as in our second case (Miki et al, 2018).

Pathologically, both reported cases are 3R-predominant tauopathies with frequent Pick bodies, although in the case 1 there is an additional widespread cortical astrocytic tau pathology with a proportion of astrocytes showing immunoreactivity with 4R-tau. As mentioned before, the closest mutation to D252V is the K257T mutation which has been

shown to have predominantly 3R-tau similar to Pick's disease (Forrest et al, 2018) as has the G389R mutation (Pickering-Brown et al, 2000).

Although we do not have segregation data from the families, two main factors argue in favour of the pathogenicity of these variants: both are novel (not present in gnomAD, other databases, or in our in-house exome sequencing data on >3000 samples including healthy controls and non-FTD diagnoses); and both have CADD scores of 21.5 indicating these are predicted to be in the top 1% of the most deleterious variations in the human genome.

In conclusion, these two novel variants are likely to be pathogenic, but the genetic analysis of other family members to establish segregation of the variants with the disease in the families would be essential to establish pathogenicity and the biological analysis of the function of the mutated protein could also provide important information regarding the role of these variants in the disease process.



**Disclosure statement**

The authors do not have any conflicts of interest.

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**References**

1. Benussi A, Padovani A, Borroni B. Phenotypic Heterogeneity of Monogenic Frontotemporal Dementia. *Front Aging Neurosci.* 2015 Sep 1;7:171.
2. Bermingham N, Cowie TF, Paine M, Storey E, McLean C. Frontotemporal dementia and Parkinsonism linked to chromosome 17 in a young Australian patient with the G389R Tau mutation. *Neuropathol Appl Neurobiol.* 2008 Jun;34(3):366-70.
3. Chaunu MP, Deramecourt V, Buée-Scherrer V, Le Ber I, Brice A, Ehrle N, El Hachimi K, Pluot M, Muraige CA, Bakchine S, Buée L. Juvenile frontotemporal dementia with parkinsonism associated with tau mutation G389R. *J Alzheimers Dis.* 2013;37(4):769-76.
4. Cash DM, Bocchetta M, Thomas DL, Dick KM, van Swieten JC, Borroni B, Galimberti D, Masellis M, Tartaglia MC, Rowe JB, Graff C, Tagliavini F, Frisoni GB, Laforce R Jr, Finger E, de Mendonça A, Sorbi S, Rossor MN, Ourselin S, Rohrer JD; Genetic FTD Initiative, GENFI. Patterns of gray matter atrophy in genetic frontotemporal dementia: results from the GENFI study. *Neurobiol Aging.* 2018 Feb;62:191-19.
5. Forrest SL, Kril JJ, Stevens CH, Kwok JB, Hallupp M, Kim WS, Huang Y, McGinley CV, Werka H, Kiernan MC, Götz J, Spillantini MG, Hodges JR, Ittner LM, Halliday GM. Retiring the term FTDP-17 as MAPT mutations are genetic forms of sporadic frontotemporal tauopathies. *Brain.* 2018 Feb 1;141(2):521-534.
6. Fumagalli GG, Basilio P, Arighi A, Bocchetta M, Dick KM, Cash DM, Harding S, Mercurio M, Fenoglio C, Pietroboni AM, Ghezzi L, van Swieten J, Borroni B, de Mendonça A, Masellis M, Tartaglia MC, Rowe JB, Graff C, Tagliavini F, Frisoni GB, Laforce R Jr, Finger E, Sorbi S, Scarpini E, Rohrer JD, Galimberti D; Genetic FTD Initiative (GENFI). Distinct patterns of brain atrophy in Genetic Frontotemporal Dementia Initiative (GENFI) cohort revealed by visual rating scales. *Alzheimers Res Ther.* 2018 May 24;10(1):46.

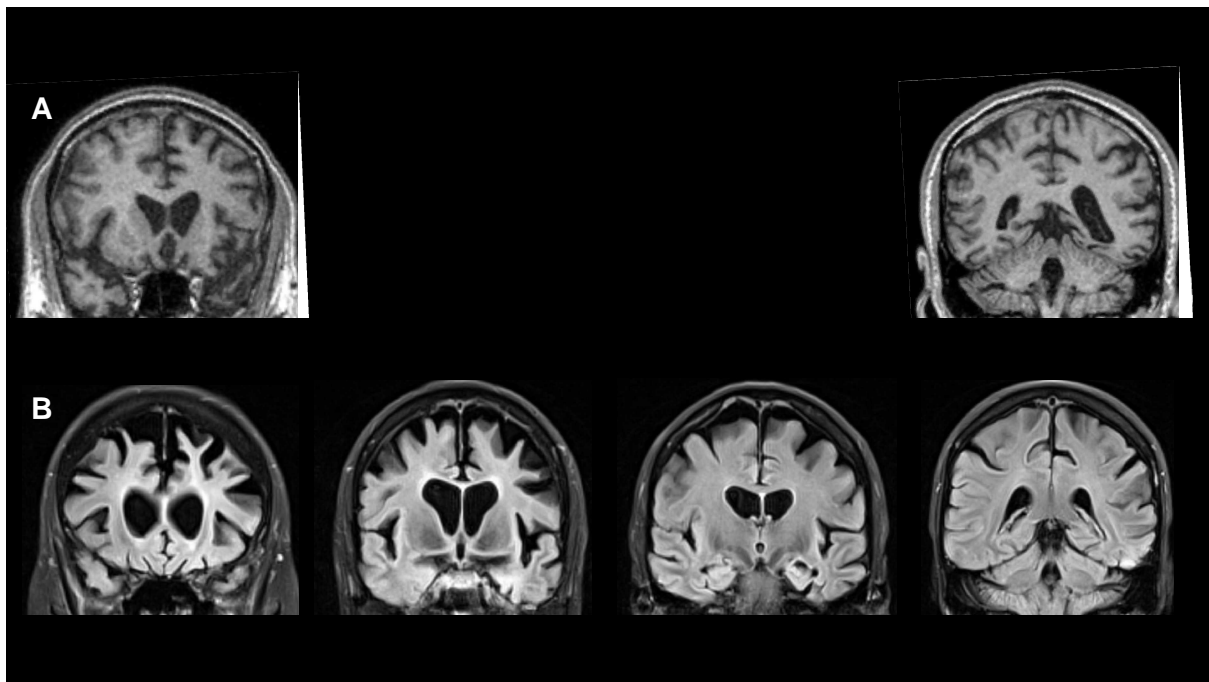
7. Ghetti B, Murrell JR, Zolo P, Spillantini MG, Goedert M. Progress in hereditary tauopathies: a mutation in the Tau gene (G389R) causes a Pick disease-like syndrome. *Ann N Y Acad Sci.* 2000;920:52-62.
8. Ghetti B, Oblak AL, Boeve BF, Johnson KA, Dickerson BC, Goedert M. Invited review: Frontotemporal dementia caused by microtubule-associated protein tau gene (MAPT) mutations: a chameleon for neuropathology and neuroimaging. *Neuropathol Appl Neurobiol.* 2015 Feb;41(1):24-46.
9. Greaves CV, Rohrer JD. An update on genetic frontotemporal dementia. *J Neurol.* 2019 Aug;266(8):2075-2086.
10. Hutton M, Lendon CL, Rizzu P, Baker M, Froelich S, Houlden H, Pickering-Brown S, Chakraverty S, Isaacs A, Grover A, Hackett J, Adamson J, Lincoln S, Dickson D, Davies P, Petersen RC, Stevens M, de Graaff E, Wauters E, van Baren J, Hillebrand M, Joosse M, Kwon JM, Nowotny P, Che LK, Norton J, Morris JC, Reed LA, Trojanowski J, Basun H, Lannfelt L, Neystat M, Fahn S, Dark F, Tannenberg T, Dodd PR, Hayward N, Kwok JB, Schofield PR, Andreadis A, Snowden J, Craufurd D, Neary D, Owen F, Oostra BA, Hardy J, Goate A, van Swieten J, Mann D, Lynch T, Heutink P. Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature.* 1998 Jun 18;393(6686):702-5.
11. Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. *Nat Genet.* 2014;46:310-5.
12. Liang Y, Gordon E, Rohrer J, Downey L, de Silva R, Jäger HR, Nicholas J, Modat M, Cardoso MJ, Mahoney C, Warren J, Rossor M, Fox N, Caine D. A cognitive chameleon: lessons from a novel MAPT mutation case. *Neurocase.* 2014;20(6):684-94.
13. Miki T, Yokota O, Takenoshita S, Mori Y, Yamazaki K, Ozaki Y, Ueno SI, Haraguchi T, Ishizu H, Kuroda S, Terada S, Yamada N. Frontotemporal lobar degeneration due to P301L tau mutation showing apathy and severe frontal atrophy but lacking other behavioral changes: A case report and literature review. *Neuropathology.* 2018 Jun;38(3):268-280.

14. Murrell JR, Spillantini MG, Zolo P, Guazzelli M, Smith MJ, Hasegawa M, Redi F, Crowther RA, Pietrini P, Ghetti B, Goedert M. Tau gene mutation G389R causes a tauopathy with abundant pick body-like inclusions and axonal deposits. *J Neuropathol Exp Neurol.* 1999 Dec;58(12):1207-26.
15. Pickering-Brown S, Baker M, Yen SH, Liu WK, Hasegawa M, Cairns N, Lantos PL, Rossor M, Iwatsubo T, Davies Y, Allsop D, Furlong R, Owen F, Hardy J, Mann D, Hutton M. Pick's disease is associated with mutations in the tau gene. *Ann Neurol.* 2000 Dec;48(6):859-67.
16. Poorkaj P, Bird TD, Wijsman E, Nemens E, Garruto RM, Anderson L, Andreadis A, Wiederholt WC, Raskind M, Schellenberg GD. Tau is a candidate gene for chromosome 17 frontotemporal dementia. *Ann Neurol.* 1998 Jun;43(6):815-25
17. Rizzini C, Goedert M, Hodges JR, Smith MJ, Jakes R, Hills R, Xuereb JH, Crowther RA, Spillantini MG. Tau gene mutation K257T causes a tauopathy similar to Pick's disease. *J Neuropathol Exp Neurol.* 2000 Nov;59(11):990-1001.
18. Rohrer JD, Ridgway GR, Modat M, Ourselin S, Mead S, Fox NC, Rossor MN, Warren JD. Distinct profiles of brain atrophy in frontotemporal lobar degeneration caused by progranulin and tau mutations. *Neuroimage.* 2010 Nov 15;53(3):1070-6.
19. Rossi G, Marelli C, Farina L, Laurà M, Maria Basile A, Ciano C, Tagliavini F, Pareyson D. The G389R mutation in the MAPT gene presenting as sporadic corticobasal syndrome. *Mov Disord.* 2008 Apr 30;23(6):892-5.
20. Rovelet-Lecrux A, Hannequin D, Guillin O, Legallic S, Jurici S, Wallon D, Frebourg T, Campion D. Frontotemporal dementia phenotype associated with MAPT gene duplication. *J Alzheimers Dis.* 2010;21(3):897-902.
21. Snowden JS, Adams J, Harris J, Thompson JC, Rollinson S, Richardson A, Jones M, Neary D, Mann DM, Pickering-Brown S. Distinct clinical and pathological phenotypes in frontotemporal dementia associated with MAPT, PGRN and C9orf72 mutations. *Amyotroph Lateral Scler Frontotemporal Degener.* 2015;16(7-8):497-505.

22. Spillantini MG, Crowther RA, Kamphorst W, Heutink P, van Swieten JC. Tau pathology in two Dutch families with mutations in the microtubule-binding region of tau. *Am J Pathol.* 1998 Nov;153(5):1359-63.
23. Tacik P, DeTure MA, Carlomagno Y, Lin WL, Murray ME, Baker MC, Josephs KA, Boeve BF, Wszolek ZK, Graff-Radford NR, Parisi JE, Petrucelli L, Rademakers R, Isaacson RS, Heilman KM, Petersen RC, Dickson DW, Kouri N. FTDP-17 with Pick body-like inclusions associated with a novel tau mutation, p.E372G. *Brain Pathol.* 2017 Sep;27(5):612-626.
24. Tolboom N, Koedam EL, Schott JM, Yaquib M, Blankenstein MA, Barkhof F, Pijnenburg YA, Lammertsma AA, Scheltens P, van Berckel BN. Dementia mimicking Alzheimer's disease Owing to a tau mutation: CSF and PET findings. *Alzheimer Dis Assoc Disord.* 2010 Jul-Sep;24(3):303-7.
25. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res.* 2010;38:e164.
26. Ygland E, van Westen D, Englund E, Rademakers R, Wszolek ZK, Nilsson K, Nilsson C, Landqvist Waldö M, Alafuzoff I, Hansson O, Gustafson L, Puschmann A. Slowly progressive dementia caused by MAPT R406W mutations: longitudinal report on a new kindred and systematic review. *Alzheimers Res Ther.* 2018 Jan 9;10(1):2.

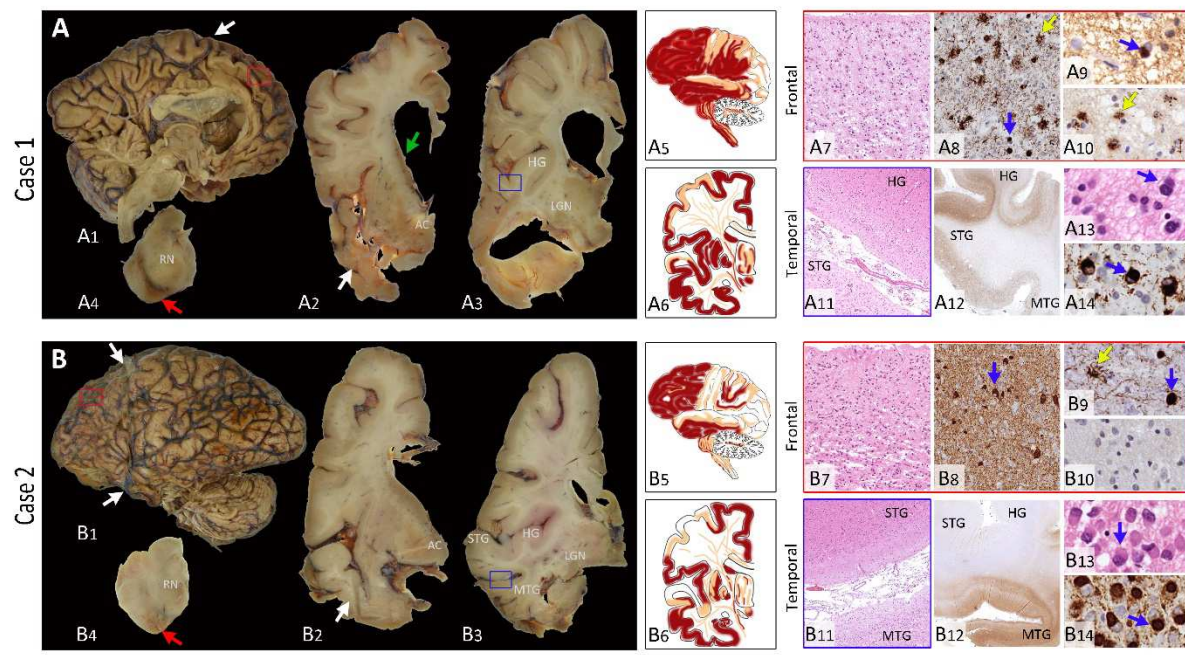
**Figures**

**Figure 1:** Magnetic resonance imaging of case 1(A) and case 2 (B) showing coronal volumetric T1 scans. Asymmetrical, left more than right, temporal greater than frontal atrophy is seen in case 1, whilst in case 2 there is relatively symmetrical bilateral frontal more than temporal lobe atrophy.

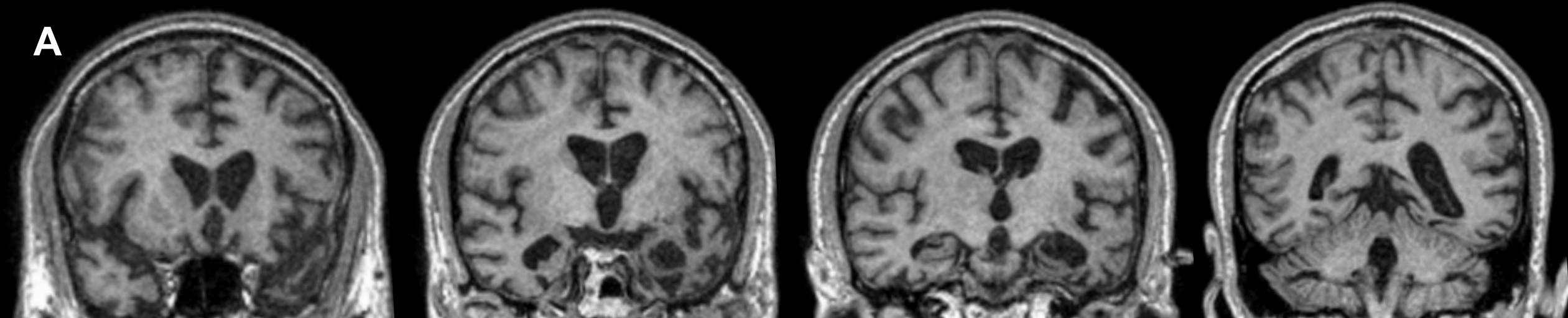
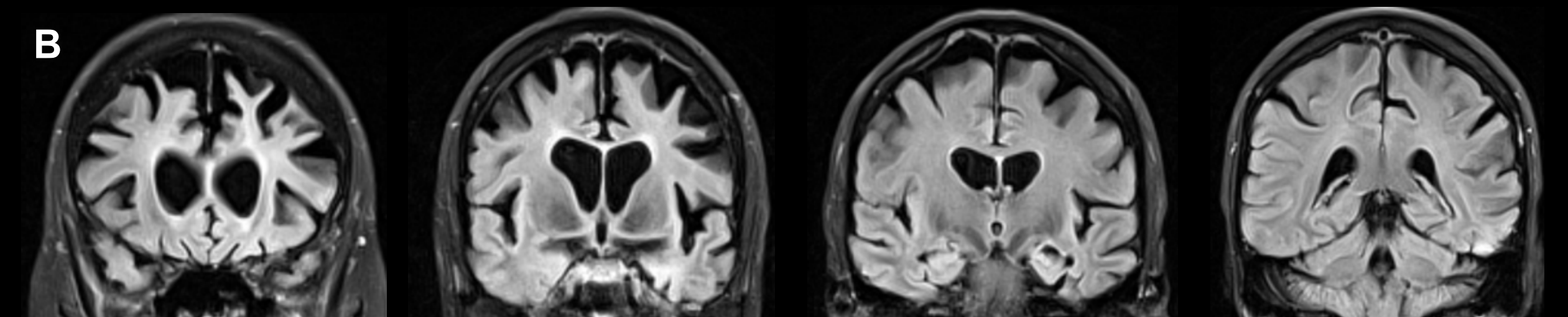


**Figure 2: Macroscopic and microscopic pathology and schematic representation of Case 1 (A1-A14) and Case 2 (B1-B14). Prominent frontal lobe atrophy is seen on the medial surface in Case 1 (A1) and on the convex lateral surface in Case 2 (B1) (sharp, knife-edge frontal lobe atrophy with some sparing of the motor cortex in both cases is highlighted with white arrows). Frontal lobe atrophy is also evident on the coronal sections of both cases (A2, A3, B2 and B3). The anterior temporal lobe at the level of the anterior commissure (AC) shows particularly severe atrophy in Case 1 (A2, white arrow), and comparably less severe medial atrophy with better preservation of the superior anterior temporal gyrus in Case 2 (B2, white arrow). At the level of the lateral geniculate nucleus (LGN) in Case 1, there is very severe atrophy of the hippocampus, parahippocampal and inferior temporal gyri, middle temporal gyrus (MTG) and superior temporal gyrus (STG), with better preservation of the Heschl's gyrus (HG) (A3). In Case 2, there is prominent atrophy of the hippocampus, parahippocampal, inferior and middle temporal gyri, with much better preservation of the STG and HG (B3). The frontal horns of the lateral ventricles are severely dilated in both cases (A1, A2, A3, B2 and B3) and the temporal horn is severely dilated in Case 1 (A3). Also the 3<sup>rd</sup> ventricles are dilated in both cases (A3 and B3). In Case 1, there is marked atrophy of the caudate nucleus (A2, green arrow) and in both cases there is globus pallidus (A2 and B2) and mild thalamus atrophy (A3 and B3). In the midbrain at the level of the red nucleus (RN) in both cases, there is conspicuous atrophy of the fronto-pontine tracts (A4 and B4, red arrows,) and pallor of the substantia nigra, which shows rusty discoloration in Case 1. Schematic representation of the extent of atrophy and tau pathology severity is demonstrated for Case 1 (A5 and A6) and Case 2 (B5 and B6, dark red represents the most severe pathology, amber scanty atrophy and tau pathology, and uncoloured corresponds to areas with preserved cytoarchitecture and absent tau pathology). Very severe pan-cortical frontal lobe atrophy with rarefaction of the neuropil and prominent gliosis is confirmed in both cases (A7 and B7, corresponding regions highlighted as red squares in A1 and B1). In Case 1, across the frontal lobe, in addition to Pick bodies (A8, blue arrow), there is widespread cortical astrocytic tau pathology, resembling ramified and tufted astrocytes (yellow arrow). Pick bodies show positive immunolabelling for 3R tau (A9, blue arrow) and a proportion of the astrocytic tau pathology is immunoreactive for 4R tau (A10, yellow arrow). In the frontal lobe of Case 2, there is a dense meshwork of tau positive neuropil threads and frequent Pick bodies (B8, blue arrow). 3R tau immunostaining accentuates Pick bodies (B9, blue arrow) and also highlights occasional astrocytic tau pathology (yellow arrow), whilst immunostaining for 4R tau (B10) is negative. In Case 1, there is severe atrophy of STG and better preserved cortical cytoarchitecture**

in HG (A11). In Case 2, MTG shows severe atrophy, but the cytoarchitecture of STG is much better preserved (B11, corresponding macroscopic regions are highlighted with blue squares in A3 and B3). Density of hyper-phosphorylated tau pathology in the medial temporal lobes of both cases reflects the degree of cortical atrophy; in Case 1 tau pathology extends into HG (A12), but in Case 2 tau pathology is minimal in the superior aspect of the STG and in HG (B12). In Case 1, there is a very severe atrophy of the dentate gyrus with tau positive Pick bodies seen in the residual granule cells (A13 and A14, blue arrows). Granule cells of the dentate gyrus in Case 2 are comparably better preserved, with numerous Pick bodies evident in the residual neurones (B13 and B14, blue arrows highlight representative inclusions). Scale bar: 300 $\mu$ m in A11 and B11, 600 $\mu$ m A12 and B12, 100 $\mu$ m in A7, A8, B7, B8, 50 $\mu$ m in A9, A10, B9, B10 and 30 $\mu$ m in A13, A14, B13 and B14.





**A****B**

**Highlights**

- **Over 60 *MAPT* mutations are currently described – we describe two novel mutations: p.D252V and p.G389\_I392del.**
- **Both novel mutations are associated with a frontotemporal dementia clinical syndrome similar to previously described *MAPT* mutations.**
- **Both novel mutations are associated with a primary 3-repeat tauopathy at post-mortem.**

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## 1. Disclosures

- a) The authors report no conflicts of interest related to this work. JDR has been a member of a Medical Advisory Board for Ionis pharmaceuticals, Wave Life Sciences, Alector pharmaceuticals and Prevail pharmaceuticals, all unrelated to this work.
- b) None of the author's institutions have contracts related to this research.
- c) No other agreements of authors or institutions are involving a financial interest in the work.

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## 3. Data in the manuscript

The data contained in the manuscript being submitted have not been previously published, nor have been submitted elsewhere, nor will be whilst under consideration here.

## 4. Consent

Consent for publication was provided by both patients through the standard approved Queen Square Brain Bank consent form.

#### **5. Authors**

All authors have reviewed the contents of the manuscript being submitted, approve the contents and validate the accuracy of the data.

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