SUPPLEMENTARY METHODS

Retire the terminology FTDP-17 as MAPT mutations are genetic forms of sporadic frontotemporal tauopathies

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Cohort

All cases were selected from a neuropathological series of cases collected by the Sydney and Cambridge Brain Banks and recruited with informed consent through their regional brain donor programs. Both programs hold ethics approval from the Human Research Ethics Committee of South Eastern Sydney Local Health District and the Universities of Sydney and New South Wales (Sydney), and Addenbrooke’s Hospital Local Ethics Committee (Cambridge) and comply with the statement of human experimentation issued by the National Health and Medical Research Council of Australia. Standardised neuropathological characterisation was performed and cases with co-existing pathologies including Alzheimer’s and Lewy body disease, and vascular pathology were excluded. Family history data were ascertained from an integrated clinical and autopsy database and by retrospective review of patient clinical files.
Tissue preparation and immunohistochemistry

Following removal at autopsy, brains were fixed in 15% neutral buffered formalin, weighed and the volume determined by fluid displacement. The cerebellum and brainstem were separated from the cerebrum. The selection of brain regions-of-interest available for all cases was based on key brain areas routinely sampled for standard FTLD neuropathological assessment included: the frontal, precentral and temporal cortices, the hippocampus at the level of the lateral geniculate nucleus, basal ganglia at the level of the head of the caudate nucleus, the midbrain at the level of the red nucleus and the pons at the level of the locus coeruleus. The anterior cingulate cortex, amygdala, subthalamic nucleus and medulla at the level of the hypoglossal nucleus, and lateral cerebellar hemisphere with dentate nucleus were also available in some cases.

Tissue blocks from each region-of-interest were embedded in paraffin wax, cut at 10 μm on a microtome and stained using routine histological (haematoxylin and eosin), silver (modified Bielschowsky (Garvey et al., 1991) and Gallyas (Kuninaka et al., 2015)) and immunoperoxidase techniques. Antibodies for immunoperoxidase staining were against phosphorylated tau (clone AT8; mouse; 1:1000; Cat. No. MN1012; Thermo Scientific, Pittsburgh, USA, performed using a Discovery XT autostainer, Ventana Medical Systems, Tuscon, AZ, USA), 3R-tau (mouse; 1:50; Cat. No. 05-803; Abcam; Cambridge, UK, performed manually) and 4R-tau (mouse; 1:50; Cat. No. 05-804; Abcam, performed manually). For antigen retrieval, 3R-tau required heating in the pressure cooker at 110°C for 30 min in Tris-EDTA buffer (pH 9.0), and 4R-tau required pre-treatment with formic acid for 15 min followed by heating in the pressure cooker at 110°C for 30 min in Tris-EDTA buffer. Following blocking of endogenous peroxidase activity in 100% methanol with 3% hydrogen peroxide for 10 min, sections were blocked in 10% normal horse serum (NHS) in Tris-buffered saline (TBS, pH 7.4). Primary antibodies diluted in TBS with 1% NHS were incubated at 37°C for one hour, followed by incubation in EnVision Dual Link Polymer (Cat. No. K4061; DAKO; Glostrup, Denmark). Dark brown staining was visualised by adding hydrogen peroxide to a 3’3’-diaminobenzidine solution. AT8, 3R-tau and 4R-tau-immunostained and Gallyas silver stained sections were counter-stained with haematoxylin.
DNA extraction and MAPT gene screening
Most FTLD-tau cases with a MAPT mutation were screened during life when a strong family history and a high likelihood of a genetic cause was identified. Remaining cases were screened for MAPT mutations by direct Sanger sequencing of polymerase chain reaction (PCR) products derived from genomic DNA. DNA was extracted from fresh frozen brains or if unavailable, from formalin-fixed paraffin embedded sections from each case. Nucleotide sequence information from each PCR product was obtained from both strands and possible mutations verified by an independent amplification of the PCR product and resequencing. The S305S MAPT mutation is assumed to be present in the second S305S sibling that came to autopsy (Halliday et al., 2006) following identification of this mutation in the two other siblings. However, frozen tissue was unavailable and intact DNA was not able to be extracted from formalin-fixed paraffin embedded sections from this case.

Analyses
All sections were viewed under a Zeiss AxioSkop microscope (Munchen-Hallbergmoss, Germany) and 8-bit images were captured with a Zeiss AxioCam HRc camera with Axiosvision 4.7 software. Assessment of neuronal loss and gliosis was made from haematoxylin and eosin or modified Bielschowsky silver-stained sections. The severity of neuropathological features immunostained with AT8 in a minimum of two cortical regions was semi-quantitatively graded on a four-point scale. The average number of balloon neurons and astrocytic plaques per field of view under 100x magnification were graded as: absent (-), mild (+; 1 – 2 inclusions), moderate (++; 3 – 9 inclusions) or severe (++++; 10+ inclusions). The average number of tufted astrocytes, globular oligodendroglial inclusions and coiled bodies in grey and white matter per field of view under 200x magnification were graded as: absent (-), mild (+; < 1 inclusion), moderate (++; 1 - 4 inclusions) or severe (++++; 5+ inclusions). Internal validation of neuropathological grading was performed by two authors on 11 cases used in the current study, and included seven cases with a mutation in MAPT (cases 2, 3, 4, 5, 6, 7, 10) and four sporadic FTLD-tau cases with CBD pathology. Interrater agreement was calculated with Kappa interrater statistics on the neuropathological grading of severity using this four-point grading scale of astrocytic and oligodendroglial inclusions, thread pathology and balloononed neurons in these cases. Overall interrater agreement on overall neuropathological grading of severity was 78% (86 out of 110 possibilities correct) and $\kappa = 0.7$, indicating substantial agreement between raters (Landis and Koch, 1997). For
figure production, no alterations were made to the captured images except for minor adjustments to brightness and contrast using the levels command in Adobe Photoshop CS6 (San Jose, CA) to best represent immunostaining when viewed directly under the microscope.

All statistical analyses were performed using SPSS Statistics version 22 (SPSS Inc., Chicago, Illinois), and $p$ values $<0.05$ regarded as statistically significant. Basic demographic data (age at symptom onset and disease duration) were assessed using Mann-Whitney U tests and all results are expressed as the mean ± standard deviation. The 95% Confidence Interval (CI) of the mean is also reported with lower and upper bounds.

References


Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics 1997;33:159-74