

## LETTER TO THE EDITOR

## Neuropathology in the S305S tau gene mutation

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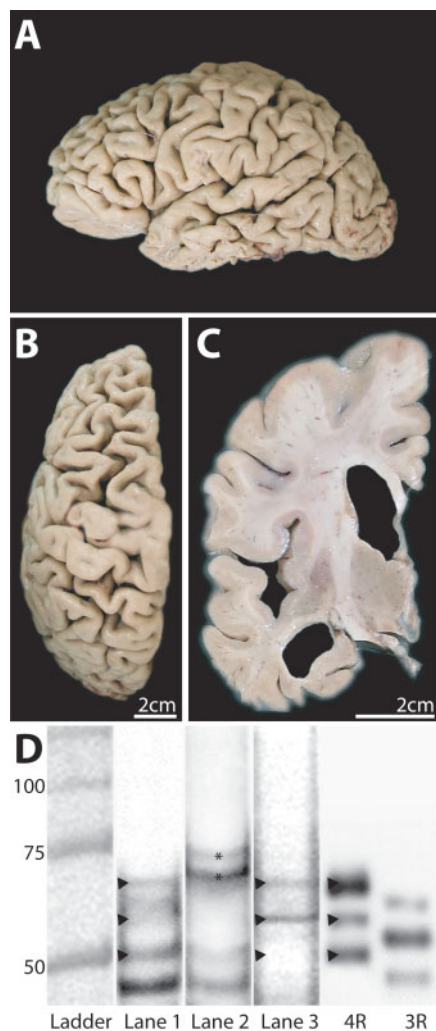
Sir,

We reported in *Brain* a novel, silent (S305S) mutation in the tau gene in two siblings and a history of presenile dementia in their mother (Stanford *et al.*, 2000). One sibling (III-15) had pathologically-proven progressive supranuclear palsy. The second affected individual (proband: III-14) has recently died at 63 years of age following 7 years of clinical symptoms, and the family consented to an autopsy for research purposes. Because of the controversy regarding the final neuropathological diagnosis in this family (Stanford *et al.*, 2001; Wszolek *et al.*, 2001) and the question raised regarding the overexpression of soluble 4R tau (Spillantini and Goedert, 2000), we would like to document the neuropathology of this second family member. The family history, initial clinical features and neuroimaging have been previously published (Stanford *et al.*, 2000).

At the time of our previous publication (Stanford *et al.*, 2000) the patient was 58 years old and was living at home with her husband. She scored 27/30 on MMSE but had deficits on neuropsychological testing and minor neurological signs. She subsequently developed obsessional behaviours such as frequent washing and was admitted to a hostel. At age 60 she scored 16/30 on MMSE; her gait was normal; she had a staring look and restricted upgaze; her speech was adynamic and dysarthric. At 61 she had a dramatic functional decline with episodes of unresponsiveness and was admitted to hospital, where a lung scan showed pulmonary emboli and she was treated with heparin and warfarin. Despite some functional improvement, she continued to have episodes of unresponsiveness, which were considered possibly epileptic in nature, although EEG was normal. Similar episodes were

reported in her mother in the 2 years before she died. III-14 was discharged to a nursing home; 2 months later she was still able to walk independently but needed major assistance with bathing, dressing and feeding and was incontinent. She scored 7/30 on MMSE; she was attentive but markedly inert and slow to answer. Eye movements were impaired and she had increased tone in the neck and upper limbs. A year later, when visited 2 weeks before she died, she was in a wheelchair, mute, with severely restricted voluntary eye movements, severe nuchal rigidity and grasp reflexes. When her arms were put in various positions by the examiner, she left them there (waxy flexibility). The picture was one of a frontal dementia (spontaneity, grasping, waxy flexibility) and progressive supranuclear palsy-like parkinsonism (facial dystonia, nuchal rigidity and supranuclear gaze palsy).

At autopsy, the brain weighed 1098 g. The right half of the brain was frozen and the left side processed and examined in the same manner as described previously for her sister (Stanford *et al.*, 2000) with the addition of immunohistochemistry staining for phosphorylated tau (AT8, 1:1000 Endogen, Woburn, MA, USA). Externally, there was mild atrophy of the convexities of the frontal and temporal lobes (Fig. 1A and B). Examination after coronal sectioning revealed ventricular dilatation, white matter loss and atrophy of the medial temporal lobe (Fig. 1C) and subthalamus. The degree and pattern of atrophy is similar to that observed in the patient's sister. Cell loss and gliosis were found in the areas of atrophy, along with silver-negative but AT8 tau-positive neurons (Fig. 2A and B). Tufted astrocytes, astrocytic plaques and ballooned neurons were observed throughout the cortex (Fig. 2A, C and D). While the hippocampus displayed



**Fig. 1** Macroscopic examination of the brain shows minimal cortical atrophy (**A** and **B**, scales equivalent), which was not prominent in either the frontal or temporal lobes. On sectioning (**C**), there was dilatation of the lateral ventricles, white matter loss and atrophy of medial temporal lobe structures. Western immunoblots (**D**) showing tau protein bands revealed mainly 4R tau (indicated by arrowheads) in the soluble and dephosphorylated insoluble fractions (Lanes 1 and 3, respectively, recombinant markers in Lanes 4R and 3R, molecular weight markers in kDa in ladder). Phosphorylated insoluble tau protein is asterisked in Lane 2.

virtually no Alzheimer-type changes, tau-positive glia were a prominent feature in this region (Fig. 2D). Sections through the basal ganglia showed numerous thickened vessel walls, many of which had calcium deposition. There was marked cell loss and gliosis in the globus pallidus and substantia nigra with most of the remaining neurons containing silver-negative but AT8 tau-positive staining (Fig. 3A and E). A high density of AT8 tau-positive structures was observed in the caudate nucleus, putamen, subthalamus, pons, oculomotor nucleus, hypoglossal nucleus, dorsal motor nucleus of the vagus nerve, ventrolateral medulla and

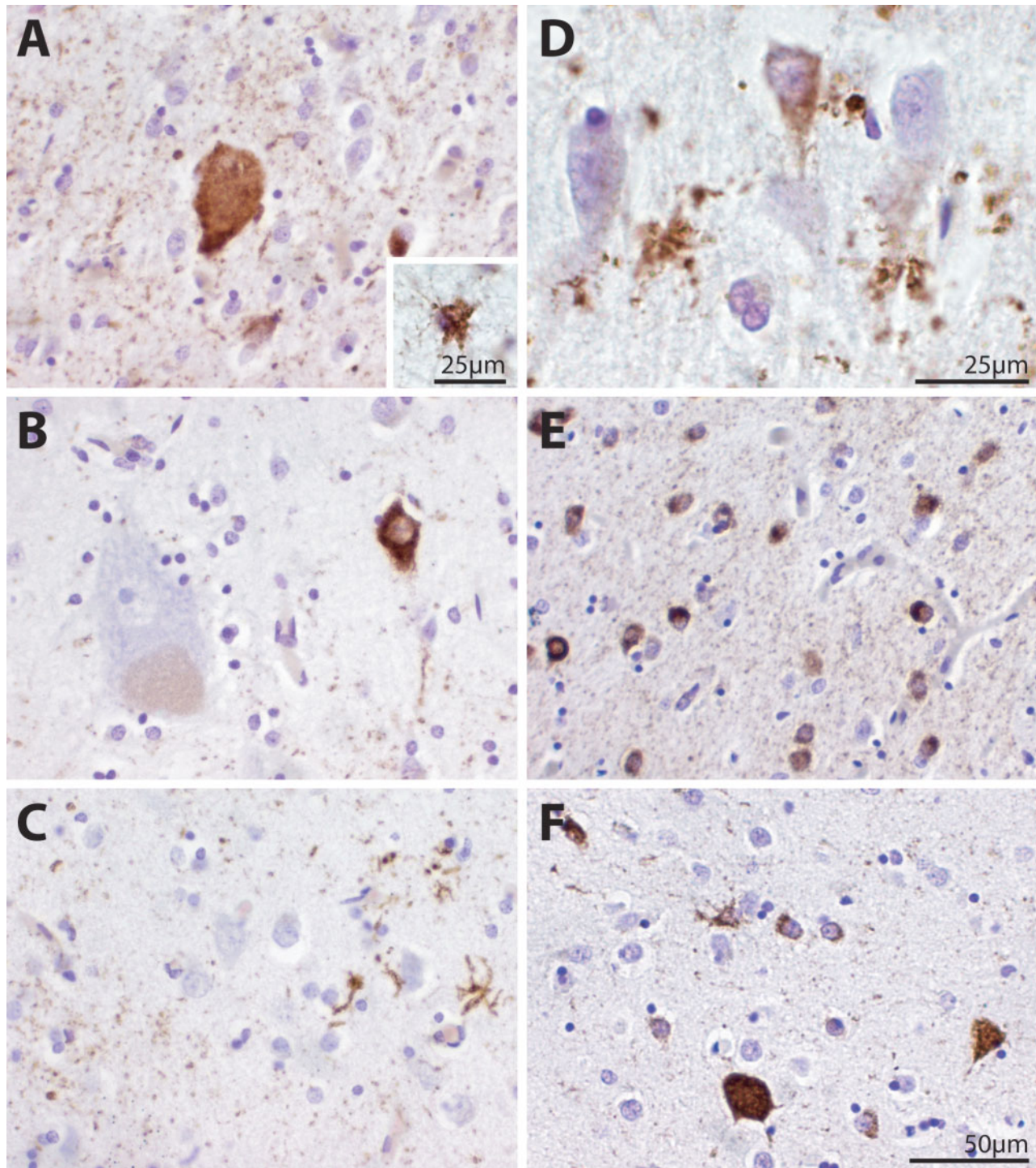
subcortical white matter tracts (Figs 2E, F and 3). In these sites neuronal loss was negligible and the vast majority of neurons and many glial cells contained AT8 phosphorylated tau. No abnormalities were found in the cerebellum. No Lewy bodies, Pick bodies, neurofibrillary tangles or neuritic plaques were observed.

Western immunoblots were performed on frozen tissue from right frontal cortex using previously described methods (Stanford *et al.*, 2003; Halliday *et al.*, 2005). Staining with phosphorylation independent tau-5 (NeoMarkers 1 : 1000, Fig. 1D Lanes 1, 2 and 3) revealed both 4 repeat (4R) and 3 repeat (3R) tau bands in the soluble protein fraction (Fig. 1D Lane 1) and two 4R tau bands (4R1N, 4R2N) in the dephosphorylated insoluble fraction (Fig. 1D Lane 3) compared with recombinant markers (Fig. 1D Lanes 4R and 3R). Phosphorylated insoluble tau protein is shown in Lane 2. The expression of soluble 4R tau in this case is similar to other cases with exon 10 intronic mutations (Spillantini and Goedert, 2000).

The neuropathological features of this case show similarities to those of her sister (limited atrophy and neuronal loss, ballooned neurons, tau-positive astrocytes) but lack the silver-positive neurofibrillary tangles. This type of pathology (AT8 tau-positive, silver-negative neurons) has been called pretangles in other cases with tau gene mutations (Iseki *et al.*, 2001; Ferrer *et al.*, 2003; Lossos *et al.*, 2003) with the same distribution pattern and clinical features observed in a case with a N296H mutation in exon 10 and 5 year history (Iseki *et al.*, 2001) as observed in III-14. However, the clinical picture in the present family is variable, with this second autopsy case having a dementia presentation (although without substantial cortical loss), in addition to many motor features (with basal ganglia cell loss). While the motor features were similar to those observed in her sister, her sister did not present with or develop dementia over the course of her disease (Stanford *et al.*, 2000). Unlike her sister, this case did not fulfil either clinical or pathological criteria for progressive supranuclear palsy and had additional pathology (astrocytic plaques and ballooned neurons), consistent with the phenotypic and pathological variation previously reported both within and between families with tau mutations (for review see Reed *et al.*, 2001). These differences suggest that significant modifying factors influence the underlying tau abnormality in these cases, as suggested in previous studies.

Our previous paper on this mutation (Stanford *et al.*, 2000) generated correspondence (Stanford *et al.*, 2001; Wszolek *et al.*, 2001) and comment in the literature particularly with respect to case diagnosis (Arvanitakis and Wszolek, 2001; Pastor and Tolosa, 2002), even though others have reported tau gene mutations associated with progressive supranuclear palsy (Pastor *et al.*, 2001; Poorkaj *et al.*, 2002) and corticobasal degeneration (Spillantini and Goedert, 2000). While all cases with tau gene mutations could be corralled under the single umbrella classification of fronto-temporal dementia with parkinsonism linked to chromosome



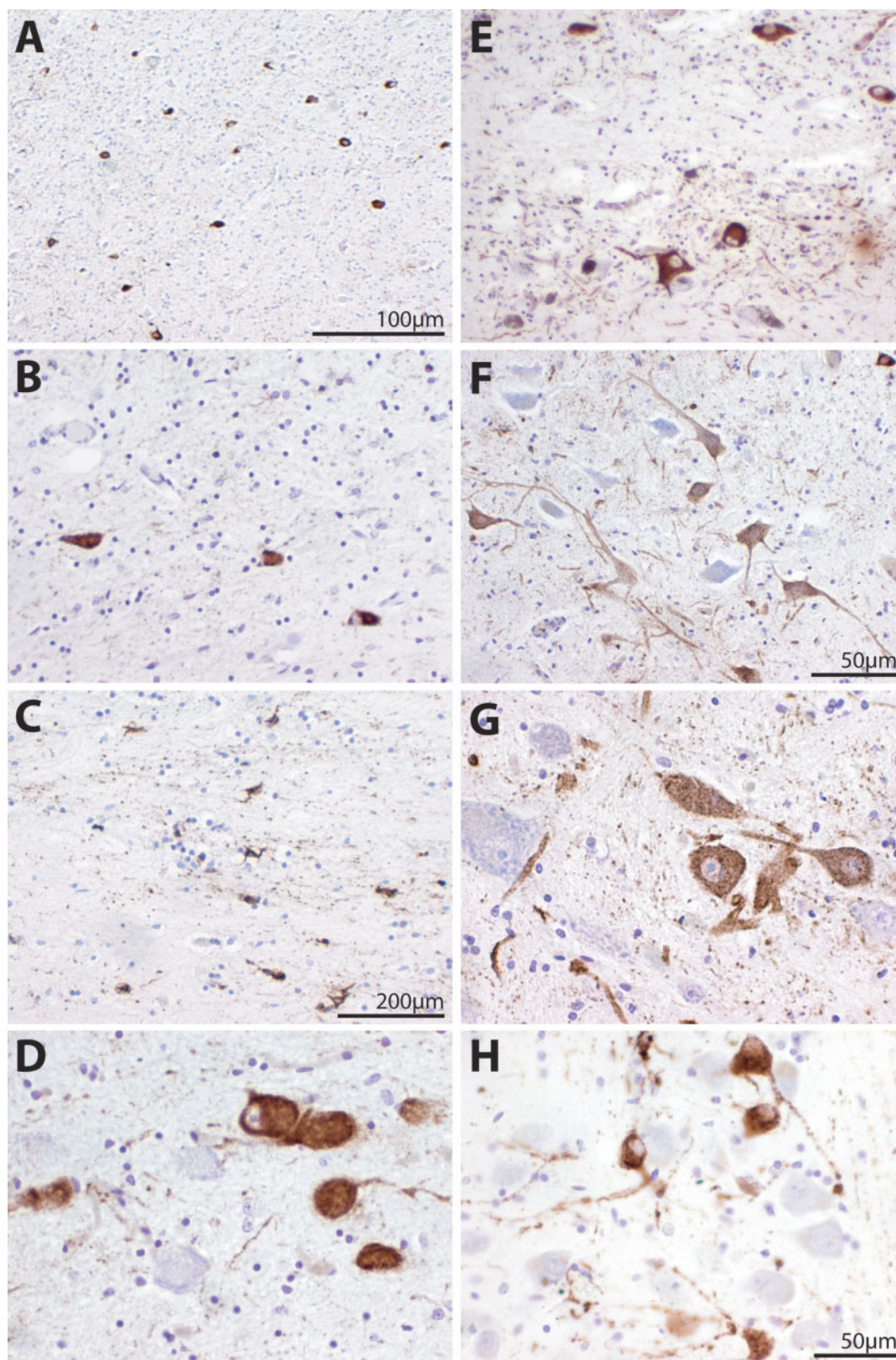


**Fig. 2** Representative photomicrographs of cortical (**A–D**, scales equivalent) and subcortical (**E** and **F**, scales equivalent) regions immunohistochemically stained for AT8 and counterstained with cresyl violet. Tau-positive ballooned neurons and tufted astrocytes (inset **A**) were found throughout the frontal cortex and putamen (**A** and **F**). AT8 tau-positive neurons (**B**) and astrocytic plaques (**C**) were found in the motor cortex. The hippocampus contained numerous AT8 tau-positive neurons and glia (**D**). The putamen contained a large proportion of tau-positive neurons (**E** and **F**).

17 (FTDP-17), understanding the potential basis for the different sporadic tauopathies (like progressive supranuclear palsy, corticobasal degeneration and Pick's disease) is unlikely to be achieved if familial forms are not identified and their

molecular differences and modifying influences understood. However, the particular case described in this letter is not like any sporadic tauopathy, and, therefore, may fit better with a diagnosis of FTDP-17.





**Fig. 3** Representative regions of the basal ganglia (**A–C**, scale in **A** equivalent to **F**) and brainstem (**D–H**, scale in **H** is equivalent for **D** and **G**, scale in **F** is equivalent for **E**) immunohistochemically stained for AT8 and counterstained with cresyl violet. Severe neuron loss was noted in the globus pallidus (**A**) and subthalamus (**B**) with the few remaining neurons tau-positive. Tau-positive astrocytes were observed throughout subcortical white matter tracts (**C**). AT8 tau deposition occurred in oculomotor (**D**), substantia nigra (**E**), dorsal motor nucleus of the vagus nerve (**F**) and hypoglossal (**G**) neurons. The pons contained a high number of tau-positive neurons, oligodendroglia and astrocytes (**H**).

## Acknowledgements

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