Frontotemporal dementia with Pick-type histology associated with Q336R mutation in the tau gene

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Summary

In this report, we describe the clinical and neuropathological features of a case of familial frontotemporal dementia (FTD), with onset at 58 years of age and disease duration of 10 years, associated with a novel mutation, Q336R, in the tau gene (tau). In vitro studies concerning the properties of tau proteins bearing this mutation, with respect to microtubule assembly and tau filament aggregation, are reported. Clinically, the patient showed alterations in memory, language and executive functions and marked behavioural change consistent with FTD, although the extent of memory impairment was more than is characteristic of FTD. At autopsy, there was degeneration of the frontal and temporal lobes associated with the presence of hyperphosphorylated tau proteins in swollen (Pick) cells and intraneuronal inclusions (Pick bodies). By immunohistochemistry, the Pick bodies contained both 3-repeat and 4-repeat tau proteins although, because no fresh tissues were available for analysis, the exact isoform composition of the aggregated tau proteins could not be determined. Neurons within frontal cortex contained neurofibrillary tangle-like structures, comprising both straight and twisted tubules, or Pick bodies in which the filaments were short and randomly orientated. In vitro, and in common with other tau missense mutations, Q336R caused an increase in tau fibrillogenesis. However, in contrast to most other tau missense mutations, Q336R increased, not decreased, the ability of mutant tau to promote microtubule assembly. Nonetheless, this latter functional change may likewise be detrimental to neuronal function by inducing a compensatory phosphorylation that may yield increased intracellular hyperphosphorylated tau species that are also liable to fibrillize. We believe the mutation is indeed pathogenic and disease causing and not simply a coincidental rare and benign polymorphism. Since this mutation is segregating with the FTD clinical and neuropathological phenotype, it has not been found in unaffected individuals and it has novel functional properties in vitro which are likely to be detrimental to neuronal function in vivo.
Introduction

After Alzheimer’s disease, frontotemporal dementia (FTD) is the second most common cause of dementia with onset before 65 years of age. The disorder is frequently (highly) familial, with ~10% cases being associated with autosomal dominant transmission due to inheritance of a mutation in the tau gene (\(\tau\)) (Mann et al., 2000). Such cases usually display variable degrees of parkinsonism in conjunction with FTD and, consequently, have become known as familial frontotemporal dementia and parkinsonism linked to chromosome 17 or FTDP-17 (Foster et al., 1997). To date, ~30 disease-associated mutations within \(\tau\) have been discovered in ~70 FTDP-17 families worldwide (for a review see Ingram and Spillantini, 2002). The \(\tau\) mutations can be grouped in various ways. Coding mutations (both missense and deletion mutations) in exons other than exon 10 disrupt the binding of tau to tubulin (Hasegawa et al., 1998); some may also increase tau aggregation (Goedert et al., 1999). Mutations in the intron after exon 10 affect the ratio of transcribed 3-repeat (3R) and 4-repeat (4R) isoforms of tau by destabilizing a stem–loop structure or disrupting splicing enhancer or silencer sequences (Hutton et al., 1998; Poorkaj et al., 1998; Spillantini et al., 1998a; Pickering-Brown et al., 2002; Taniguchi et al., 2004). Coding mutations in exon 10 reduce the ability of mutant (4R) tau isoforms to interact with microtubules (Hasegawa et al., 1998; Hutton et al., 1998; Poorkaj et al., 1998) and promote the aggregation of mutant 4R species into fibrillary structures (von Bergen et al., 2001). Nonetheless, all FTDP-17-associated \(\tau\) mutations lead to the intracellular accumulation of insoluble aggregates of tau, and for this reason the disease associated with these known mutations has become known as a ‘tauopathy’, along with other disorders such as progressive supranuclear palsy, corticobasal degeneration and Alzheimer’s disease in which intracellular, filamentous tau deposits are also present.

However, there is much pathological heterogeneity within FTDP-17 with respect to the molecular (tau isoform) composition and fine structure of the insoluble tau aggregates according to the particular mutation present. Splice site and coding mutations in exon 10 generally lead to the formation of neurofibrillary tangle (NFT)-like structures within neurons and glial cells that have the ultrastructural characteristics of wide, flat, twisted ribbons composed principally of 4R tau (Spillantini et al., 1998a; Pickering-Brown et al., 2002; but see Kobayashi et al., 2003a). On the other hand, certain mutations in exon 9 [K257T (Pickering-Brown et al., 2000; Rizzini et al., 2000), L266V (Kobayashi et al., 2003b; Hogg et al., 2003) and G272V (Spillantini et al., 1998b)], exon 10 (Kobayashi et al., 2003a), exon 11 [L315R (van Herpen et al., 2003) and S320F (Rosso et al., 2002)], exon 12 [E342V (Lippa et al., 2000) and K369I (Neumann et al., 2001)] and exon 13 [G389R (Murrell et al., 1999; Pickering-Brown et al., 2000)] are associated with a Pick-type of histology with rounded intraneuronal inclusions (Pick bodies) and swollen chromatolytic nerve cells (Pick cells) in which the insoluble tau extracted from brain can be predominantly 3R isoforms or a mix of 3R (mostly) and 4R isoforms. Curiously, certain other mutations in exon 12 (V337M) (Poorkaj et al., 1998) and exon 13 (R406W) (Hutton et al., 1998) lead to accumulation of NFT in neurons, but not in glia, of a type identical to the paired helical filaments of Alzheimer’s disease (Spillantini et al., 1996; Saito et al., 2002) composed of equivalent amounts of both 3R and 4R isoforms (Spillantini et al., 1996). Moreover, another recently reported tau mutation in exon 12 (S352L) is associated with a respiratory failure phenotype and a (probably) recessive mode of inheritance; in this instance, amorphous insoluble tau deposits were seen within neurons of the hippocampus, cerebellar nuclei and brainstem, but not cerebral cortex (Nicholl et al., 2003). The reason for this diversity of tau pathological picture is not known, but may reflect these variations in tau isoform composition, or the physicochemical properties of the accumulated (mutant) tau molecules.

Here we present a case of FTD associated with a novel tau mutation, Q336R, with Pick-type histological features. The Q336R mutation is pathogenic and increases in vitro the capacity of tau to form fibrils. However, in contrast to most other missense tau mutations reported so far, Q336R increases the ability of tau to interact with microtubules. It is curious that this particular mutation is in the adjacent codon in tau to a previously described mutation, V337M (Poorkaj et al., 1998), which reduces the ability of tau to interact with microtubules and which is associated not with Pick-type tau histology but with Alzheimer’s disease-like NFTs (Spillantini et al., 1996).

Materials and methods

Family history

The proband was a 68-year-old man who died following a 10 year history of changes in memory, language, behaviour and personality. There was a family history of a similar dementing disorder, consistent with autosomal dominant transmission, in the proband’s father, paternal grandfather and two paternal great uncles. The
proband’s father was described as an eccentric recluse, with long-standing memory problems, who neglected himself in his later years and had a similar personality to the proband. There was no post-mortem. The proband’s paternal grandfather had memory loss in his later years, died in his 70s and showed cerebral degeneration at post-mortem. The latter’s two brothers were reported to have had dementia. Little is known about their parents.

Pathological methods
The entire brain of the proband was fixed by immersion in 10% buffered formalin for 4 weeks prior to examination and sectioning. Representative tissue blocks were cut from cerebral cortical and other regions to include frontal, temporal, cingulate, parietal and occipital cortex, hippocampus, amygdala, basal ganglia, substantia nigra, brainstem and cerebellar cortex. Tissue blocks were routinely processed into paraffin wax and sections cut at a thickness of 6 μm. Sections were stained by routine neurohistological methods, including Weigert’s haematoxylin–eosin and Luxol Fast Blue, and immunohistochemically using a standard avidin–biotin–peroxidase method. For the immunodetection of tau, primary antibodies Tau (Dako, Ely, UK, 1:500), AT8 (Ser202/Thr205; 1:200), AT180 (Thr231; 1:200), AT270 (Thr181; 1:200) (all from Innogenetics, Belgium), CP13 (Ser202) and PHF-1 (Ser396/404) (gift of Dr P. Davies, 1:2000 and 1:1000, respectively), 12E8 (Ser262) (gift of Dr P. Seubert, 1:200) and the 3R (RD3) and 4R (RD4) tau-specific monoclonal antibodies (de Silva et al., 2003) (1:3000, RD3; 1:100, RD4) were used. Sections stained by the latter antibodies required pressure cooker pre-treatment (5 min in sodium citrate buffer, 94°C for 30 s, 60–50°C ‘touchdown’ annealing for 30 s, and 72°C for 45 s. The final extension cycle was 72°C for 10 min. Products were purified using the Multiscreen filtration system (Millipore, Bedford, CA). Each exon was sequenced on an ABI3100 Genetic Analyzer, using the relevant PCR primers and Big Dye Terminator Cycle Sequencing chemistry (version 3.1, Applied Biosystems, CA). The Q336R mutation was confirmed by MscI digestion of the exon 12 PCR products; the mutation eliminates an MscI site.

One hundred control individuals were screened for the Q336R change by PCR–restriction fragment length polymorphism (RFLP) analysis with the MscI assay.

Microtubule assembly
Site-directed mutagenesis was performed to change Q336 to arginine (in the numbering of the 441 amino acid isoform of human tau) in the 3R, 381 amino acid isoform (T37), and in the 4R, 412 amino acid isoform (T46) of human tau (Hasegawa et al., 1998), using a site-directed mutagenesis kit (Stratagene). All constructs were verified by DNA sequencing. Wild-type and mutated tau proteins were expressed in Escherichia coli BL21 (DE3), purified, and microtubule assembly assay was performed as described previously (Hasegawa et al., 1998). All experiments were repeated three times and the results expressed as mean (percentage of wild-type) ± SEM. The concentrations of wild-type and mutant tau proteins employed in each experiment were identical, as checked by high-performance liquid chromatography (HPLC), protein sequencing and Coomassie staining.

Tau filament assembly
Purified wild-type or 3R (T37) (8 μM) and 4R tau (T46) (8 μM) forms of Q336R mutant human tau were incubated in the presence of 100 μg/ml heparin in 50 μl of 30 mM Tris–HCl pH 7.5, 0.1% NaN3 at 37°C for 72 h. Aliquots were placed on grids and stained with 2% sodium phosphotungstate. Micrographs were recorded at a nominal magnification of ×20 000. For quantification, tau filaments were suspended in four volumes of 30 mM Tris–HCl buffer pH 7.5 containing 1% Sarkosyl and precipitated by ultracentrifugation at 50 000 r.p.m. for 20 min. The Sarkosyl-insoluble tau was denatured with SDS sample buffer, separated by SDS–PAGE and stained with Coomassie brilliant blue. The tau bands were quantified with NIH-Image. All experiments were repeated three times. As above, the starting concentrations of wild-type and mutant tau proteins employed in each experiment were identical.

Results
Clinical findings
The proband, a craftsman, presented at 62 years with a 4 year history of failing memory. He reported problems with immediate recall and several times he had become lost in his locality. He had decreased concentration and found it increasingly difficult to continue his trade. His wife regarded him as less tolerant and more demanding. On examination, he had MSQ seven out of 10. He had difficulty recalling a name

Genomic DNA was extracted from peripheral blood samples by routine methods. tau exons 7 and 9–13 were amplified by polymerase chain reaction (PCR) using primers designed to flanking intronic sequence. Each 50 μl PCR contained 25 ng of DNA template, 20 pmol of each primer, 0.2 mM dNTPs and 1 U of Taq polymerase (Qiagen, CA). Cycling conditions were 35 cycles of 94°C for 30 s, 60–50°C ‘touchdown’ annealing for 30 s, and 72°C for 45 s. The final extension cycle was 72°C for 10 min. Products were purified using the Multiscreen filtration system (Millipore, Bedford, CA). Each exon was sequenced on an ABI3100 Genetic Analyzer, using the relevant PCR primers and Big Dye Terminator Cycle Sequencing chemistry (version 3.1, Applied Biosystems, CA). The Q336R mutation was confirmed by MscI digestion of the exon 12 PCR products; the mutation eliminates an MscI site.

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and address immediately and had no recollection of it after a
delay. A CT scan was unremarkable. EEG was normal, as was
lumbar puncture. A clinical diagnosis of familial Alzheimer’s
disease was suspected.

Six months later, he was noted to be mildly disinhibited
and distractible, with poor self-monitoring. He was pedantic,
verbose and circumlocutory. Neuropsychological evaluation
revealed his MSQ to be unchanged at seven out of 10. He
could not give the precise date, his own age or the name of the
previous Prime Minister, although was otherwise oriented in
time and place. He named only two out of 30 items on the
Graded Naming test, improving to 14 out of 30 with time,
suggesting inefficient word retrieval. Language comprehen-
sion, measured by the Token test, was accurate but slowed.
He wrote with a large, expansive script, with spelling errors.
He could sign his name. Spatial and visuo-constructional
tasks were performed well. Performance on the Trail
making attentional test was satisfactory. He performed
poorly on a list learning task, showing an absence of learning
curve over successive trials. Performance on a frontal
executive test showed mild difficulties. His verbal fluency
and Wisconsin Card Sorting Test scores were satisfactory,
although the latter was achieved only after much agonizing
and delay. The clinical picture of impaired social functioning,
mild executive deficits, evolving dysphasia and poor
memory, in the context of preserved perceptual and spatial
skills, suggested frontotemporal lobe dysfunction, with
relative preservation of function of the posterior cerebral
hemispheres.

He was reviewed at regular intervals over the next 4 years.
There was a marked deterioration in his social conduct. He
became increasingly overfamiliar and disinhibited, lacking in
self-care and concern for others. Unconcerned jocularity
gradually gave way to belligerence, verbal aggression and
paranoia, particularly directed towards his wife. His beha-

He was admitted to long-term care but deteriorated
rapidly, dying at the age of 68 years, following a total
duration of illness of 10 years.

**Neuropathological findings**

External examination of the brain showed moderately severe
atrophy of the frontal lobes and severe atrophy of the anterior
temporal lobes, the latter being worse on the right- than on the
left-hand side. The posterior parietal and occipital lobes were
only mildly affected. The left cerebral hemisphere weighed
470 g fresh, the right cerebral hemisphere weighed 461 g and
the brainstem and cerebellum weighed 171 g (total weight
1102 g).

There was marked dilatation of the lateral ventricle and the
temporal horn extension. On section, there was severe
atrophy of the medial temporal lobe structures (both
hippocampus and amygdala) and the inferior temporal
gyrus; the middle and superior temporal gyri were less
affected, especially posteriorly (Fig. 1). The anterior temporal
lobe was soft and the distinction between grey and white
matter lost, the latter having a brownish coloration. The
frontal and anterior parietal lobes were moderately atrophic,
but here the distinction between grey and white matter was
well maintained (Fig. 1). The basal ganglia were smaller than
usual but without other significant abnormality. The corpus
callosum appeared normal. The substantia nigra was under-
pigmented.

Varying degrees of cerebral cortical neuronal loss and
reactive astrogliosis were seen, these principally affecting the
frontal and anterior inferior and middle temporal gyri, with
relative sparing of the superior temporal gyrus. In severely
affected areas, neuronal loss was subtotal and astrogliosis severe, leading to widespread spongiosis and loss of cytoarchitecture. Many remaining neurons, mostly the small and large pyramidal cells of layers II and III, to a lesser extent those of layers V and VI, showed rounded inclusions intensely reactive with AT8 antibody (Fig. 2B). Other neurons in deeper layers of frontal and temporal cortex were swollen and chromatolytic, being strongly positive with AT270 antibody (Fig. 2A). Nerve cells containing more amorphous, often peripherally located, tau deposits were also widespread (Fig. 2B), as were tau-positive neuropil threads within grey matter. Occasional tau-immunoreactive astrocytes were seen within the cerebral cortical grey matter, mostly in layer III in motor cortex. Occasional tau-immunoreactive astrocytes and fibrillary structures resembling axons were seen in deep white matter. Similar, but less severe, changes to those seen in frontal and temporal cortex were present in anterior parietal and motor cortical regions.

In the hippocampus, there was severe loss of pyramidal cells from areas CA1, CA2 and CA3, less so from CA4/5. There were many AT8-immunoreactive spherical inclusions within the pyramidal cells of the CA regions and subiculum of the hippocampus (Fig. 2C). There were also many intensely stained, intracellular NFTs within pyramidal cells of CA regions and subiculum and many less strongly stained, more loosely aggregated, extracellular tangles (Fig. 2C), often adjacent to those containing the rounded inclusions. Extracellular tangles were GFAP positive and associated with reactive astrocytes (Fig. 2D). Spherical inclusions were also conspicuous in the granule cells of the dentate fascia (Fig. 2E and F). There was virtually complete loss of the stellate nerve cells of layer II of the entorhinal cortex with only occasional extracellular tangle-like cells remaining.

There were occasional amorphous tau-immunostained cells, and some intracellular rounded inclusions in the caudate nucleus and nucleus accumbens, but these were rare in the putamen. There were scattered tau-immunoreactive neurons throughout the thalamus, but mostly with a neurofibrillary appearance. In the nucleus basalis of Meynert, there were many tau-immunoreactive neurons, some containing amorphous tau deposits, while in others rounded or (more usually) globose NFT-like structures were seen, some of which were extracellular. Inclusions were also present in neurons of the substantia nigra and locus coeruleus, associated with moderate neuronal loss and extracellular neuromelanin pigment; neuropil threads were widespread within the substantia nigra (Fig. 2H). Inclusions were widespread within the dorsal and medial raphe. There were no tau-immunoreactive glial cells within the internal capsule or corticostriatal tracts, nor were nerve cells of the pontine and dentate nuclei tau immunoreactive. The neuronal inclusions, swollen nerve cells and affected glial cells were all strongly immunoreactive with AT8 (Fig. 2B, C and E), AT180, AT270 (Fig. 2A), CP13 and PHF-1 antibodies, and also reactive for both RD3 (3R) (Fig. 2F) and RD4 (4R) (Fig. 2G) tau-specific antibodies. Possibly because of differing antibody affinities, neither RD3 nor RD4 antibodies detected an equivalent level of tau pathology; indeed RD4 antibody labelled less tau pathology than RD3. Although Pick bodies were labelled with RD3 and, to a lesser extent, RD4 antibody, it was not possible (again because of differing antibody affinities) to determine conclusively whether the same Pick body contained both 3R and 4R protein in co-localization, or whether there were separate subsets of Pick bodies containing either 3R (more often) or 4R (less often) tau proteins. The inclusions generally were only weakly, or non-reactive with 12E8 antibody, though occasional cortical nerve cells and those in the hippocampus, nucleus basalis, substantia nigra, locus coeruleus and raphe nuclei, where the tau protein was aggregated into distinct neurofibrillary structures, showed stronger 12E8 immunoreaction (not shown). The rounded inclusions and the swollen cells were both only weakly, or non-reactive with anti-ubiquitin antibodies, though the intracellular NFT-like structures were often strongly ubiquitinated (not shown). Immunohistochemistry for α-synuclein revealed neither cortical nor nigral Lewy bodies. Neither neuritic plaques nor deposits of amyloid β protein were present. There was a moderately severe arteriosclerosis within the deep white matter of cerebral cortex with moderate to severe loss of myelin and reactive astrogliosis.

**Electron microscopy**

Some neurons within the hippocampus contained NFT-like structures (Fig. 3A) comprising straight and twisted tubules (Fig. 3B), with many more straight than twisted tubules being present, that were immunodecorated with tau–gold particles. Other inclusions, however, resembled Pick bodies (Fig. 3C) in which the tau-immunopositive filaments were short and randomly orientated (Fig. 3D).

**Genetic analysis**

Sequence analysis of the coding and flanking intronic regions of the tau gene revealed a transitional A to G base change in exon 12 at nucleotide position 1007, resulting in a novel glutamine to arginine, or Q336R, change at the amino acid level (not shown). The presence of the mutation was confirmed by PCR–RFLP. The mutation was absent in 100 controls, checked by PCR–RFLP.

**Microtubule assembly**

Recombinant 3R (T37) and 4R (T46) human tau isoforms with the Q336R mutation were expressed, and their ability to promote tubulin assembly examined. When compared with wild-type tau, both 3R and 4R Q336R tau isoforms showed an increased ability to promote microtubule assembly (Fig. 4A and C). The increase was ~40%, when expressed as the optical density at 1.5 min (Fig. 4B and D). Such findings contrasted with those obtained with V337M tau, which, consistent with our previous findings (Hasegawa et al., 1998),
shows a moderately reduced ability to promote microtubule assembly (Fig. 4). Although microtubule binding was still greater with both 3R and 4R Q336R tau isoforms, over wild-type tau, at the end-points of the assay, the increase was not statistically significant.

**Tau filament assembly**

There was increased heparin-induced assembly of filaments using both 3R (T37) and 4R (T46) (Fig. 5) Q336R mutant tau proteins compared with wild-type tau, both when viewed electron microscopically (Fig. 5A versus B, and E versus F, respectively) or as Coomassie brilliant blue staining of the Sarkosyl-insoluble assembled tau (Fig. 5C and G, respectively) separated by SDS–PAGE. Quantification of the Sarkosyl-insoluble tau bands (Fig. 5D and H, respectively) showed increases in the amount of insoluble tau (filament assembly) using Q336R mutant tau proteins. Indeed, the effect of the mutation appeared greater in the case of mutant 3R Q336R tau where there was a 3- to 4-fold increase in filament formation (Fig. 5D) compared with mutant 4R Q336R tau where the increase was only 1.5- to 2-fold (Fig. 5H). There were no differences between levels of soluble mutant or wild-type tau.

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**Fig. 2** The temporal cortex shows severe loss of nerve cells with astrocytosis leading to loss of normal cytoarchitecture with swollen nerve cells (AT270 antibody) (A) and intraneuronal inclusions (AT8 antibody) (B). There is also severe loss of pyramidal nerve cells from CA1 and subiculum of hippocampus, with many AT8-positive, rounded inclusions and NFT-like structures both in remaining cells and extracellularly (C), the latter appearing to be strongly GFAP immunoreactive (D). Pick bodies in dentate gyrus granule cells of hippocampus are strongly immunoreactive with AT8 (E), and 3R (RD3)- (F) and 4R (RD4)- (G) specific tau antibodies. In the substantia nigra, many tau-positive NFTs and neuropil threads are present (H). Immunoperoxidase–haematoxylin. Magnification ×200. This figure can be viewed in colour as supplementary material at Brain Online.

**Fig. 3** Electron microscopic appearance of inclusion bodies in neurons of the hippocampus. NFT-like structures (A and B) are composed of mostly straight, but some twisted, tubules, whereas Pick bodies (C and D) are a random mix of short filaments. Standard electron microscopic staining (A and C), and immunogold staining (B and D).
following fibrillization (not shown). This is probably because only a small proportion of either mutant or wild-type tau forms filaments and hence, although the change in levels of insoluble tau between mutant and wild-type tau can be detected, differential effects on soluble levels are too small to determine.

The effects of the Q336R mutation on the ability of 3R (T37) and 4R tau (T46) to promote microtubule assembly (A and C) and polymerization of tubulin induced by wild-type T37, T37 Q336R and T37 V337M (A) and by wild-type T46, T46 Q336R and T46 V337M (C) as monitored by turbidity at 350 nm (B and D). Optical densities representing the extent of microtubule formation for wild-type T37, T37 Q336R and T37 V337M (B) and for wild-type T46, T46 Q336R and T46 V337M (D) at 1.5 min (expressed as a percentage of wild-type taken as 100%). Results are expressed as means ± SEM (n = 3).

The representative fields of filaments assembled from wild-type (A) and Q336R mutant 3R tau (T37) proteins (B) and wild-type (E) and Q336R mutant 4R tau (T46) proteins (F). Bar: 200 nm. Coomassie brilliant blue staining of the Sarkosyl-insoluble assembled T37 (C) and T46 (G) tau proteins separated by SDS–PAGE. Representative results of one set of experiment [three wild-type (lanes 1–3) and three mutant tau (lanes 4–6)] are shown. Quantification of the Sarkosyl-insoluble T37 (D) and T46 (H) tau bands. Results are expressed as means (expressed as a percentage of wild-type taken as 100%) ± SEM; three sets of separate experiments were performed, total samples n = 9. **P < 0.001.
Discussion
In this present study, we report on a case of familial FTDP-17 associated with a novel mutation in tau, Q336R. It is highly likely that this mutation is pathogenic since it segregates with the FTD clinical phenotype and this genetic variation has not been found in 100 unaffected individuals. This present case extends the range of mutations in tau associated with FTDP-17, particularly those that lead to the development of Pick-type histology. This type of histology has been reported most frequently in association with tau mutations in exon 9 [K257T (Pickering-Brown et al., 2000; Rizzini et al., 2000), L266V (Kobayashi et al., 2003b; Hogg et al., 2003) and G272V (Spillantini et al., 1998)], exon 10 (Kobayashi et al., 2003a), exon 11 [L315R (van Herpen et al., 2003) and S320F (Rosso et al., 2002)], exon 12 [E342V (Lippa et al., 2000) and K369I (Neumann et al., 2001)] and exon 13 [G389R (Murrell et al., 1999; Pickering-Brown et al., 2000), although interestingly, in a further case of FTDP-17 associated with the exon 9 mutation I260V, the pathological tau species deposited in the brain did not take the form of either Pick-like bodies or NFTs (Grover et al., 2003), nor did the insoluble tau adopt either of these pathological forms in the recessively inherited S352L mutation (Nicholl et al., 2003). The present case shared many of the symptoms seen in certain cases with the aforementioned tau mutations in exon 11 and elsewhere, where an early memory deficit reminiscent of Alzheimer’s disease was noted, reflecting perhaps the unusually [as compared with sporadic forms of FTD associated with Pick-type histology (Mann and South, 1993; Mann et al., 1993)] severe involvement of the medial temporal lobe structures [e.g. K257T (Pickering-Brown et al., 2000; Rizzini et al., 2000), S320F (Rosso et al., 2002), G342V (Lippa et al., 2000) and K369I (Neumann et al., 2001) tau mutations]. Nonetheless, other cases of FTDP-17 with tau mutations and Pick-type histology manifest fairly typically with personality and behavioural changes associated with a more characteristic bilateral frontotemporal atrophy [e.g. I266V (Kobayashi et al., 2003b; Hogg et al., 2003), L315R (van Herpen et al., 2003) and G389R (Murrell et al., 1999; Pickering-Brown et al., 2000)] mutations. Interestingly, and in contrast to most cases of FTDP-17 with tau splice site and exon 10 coding mutations (Reed et al., 2001; Pickering-Brown et al., 2002), none of the aforementioned cases of FTDP-17 with tau mutations and Pick histology show marked parkinsonian signs, and the substantia nigra is usually relatively well preserved. Perhaps even more puzzling is the conjunction in this case of Alzheimer- and Pick-type tau pathologies in neurons of the hippocampus, this frequently involving neighbouring cells. This has not been reported previously in other cases of FTDP-17 with tau mutations and Pick-type histology. It is possible that both Pick- and NFT-type pathologies can be driven by this particular tau mutation, but this would not explain why such overlaps were confined to the hippocampus. Given the observations that extracellular NFTs were present (as well as intracellular NFTs), whereas Pick bodies were entirely intracellular, it is possible that the Alzheimer-type pathology is coincidental, occurring at an earlier point in the disease course and this was perhaps responsible for the initial clinical symptoms of memory loss. In support of this, it was noted that many of the extracellular tangles were immunodecorated by GFAP antibody, indicating the infiltration and ‘wrapping’ of such tangles by astrocyte processes, a change commonly seen in patients with severe Alzheimer’s disease and widespread extracellular tangles in the hippocampus (Probst et al., 1982). Moreover, the involvement of the nucleus basalis, locus coeruleus and raphe nuclei predominantly by Alzheimer-type, rather than Pick-type, pathology would be consistent with this argument, since these regions are also among those particularly susceptible to NFT formation in early Alzheimer’s disease and ageing. Such Alzheimer-type pathological processes within the hippocampus might have become overwhelmed at a later stage by the Pick-type changes driven, presumably, by the tau mutation itself.

In contrast to other tau mutations in exons 9, 11, 12 and 13, the Q336R mutation has novel functional properties in vitro, leading on one hand to an increase in the potential for the mutant tau protein, especially 3R tau isoforms, to aggregate into fibrils, and on the other to an increased ability to promote microtubule assembly. Both of these effects are likely to be detrimental to neuronal function in vivo and support the view that the genetic change is indeed pathogenic and disease causing, and not simply a rare and benign polymorphism.

Although the mutation occurs in a highly conserved region of the microtubule-binding domain of tau, and previous experimental work shows that many missense mutations in such conserved regions of tau reduce the ability of tau to promote microtubule assembly and some also facilitate the fibrilization of tau (Hasegawa et al., 1998; Goedert et al., 1999), Q336R seemingly, and paradoxically, increases tau-induced microtubule assembly, presumably reflecting increased microtubule binding. Several positively charged residues have been shown to strongly influence the ability of tau to promote microtubule assembly. The Q336R mutation involves a change to a positively charged residue in the repeat region, which binds to microtubules, and so this particular mutation may enhance the affinity of tau for tubulin, thereby resulting in its increased ability to promote microtubule assembly. Although we previously have reported a slightly increased microtubule binding capability of tau with the S305N mutation (Hasegawa et al., 1999), it remains unknown whether this effect contributes to the pathogenesis of FTDP-17 in this particular instance, because the S305N mutation has such a strong (and perhaps overriding) effect on mRNA splicing, increasing exon 10 inclusion and the proportion of 4R tau (Hasegawa et al., 1999). Interestingly, and in contrast to other tau mutations in and around exon 10, the S305N mutation, like Q336R, is also associated with Pick-type histology (Kobayashi et al., 2003b) rather than the NFT-like pathology (Pickering-Brown et al., 2002). The Q336R mutation is, therefore, the second example of an...
FTDP-17 mutation that produces an increased ability of tau to assemble microtubules. Appropriate activities, and correct levels of tau in relevant cellular compartments, may be essential for proper neuronal function. Too much tau microtubule-binding activity may be just as bad for nerve cells as too little, by reducing microtubule dynamics and the ability of the neuron to respond to stress. This might explain the increased presence of phosphorylated, insoluble tau in the brain, per se, in this Q336R mutation case. In addition, the extra effect of Q336R of enhancing tau filament assembly may accelerate the aggregation of available hyperphosphorylated tau. Alternatively, the effect of this mutation on microtubule binding may be irrelevant to the pathogenic mechanism that is driven by enhanced aggregation of Q336R tau alone. In this latter hypothesis, the enhanced aggregation of Q336R tau simply leads to the formation of tau neuronal inclusions in patients with this mutation, with resultant neurodegeneration.

The missense mutations in exons 9 (K257T, L266V and G272V), 11 (L315R and S320F), 12 (Q336R and G342V) and 13 (K369I and G389R) of tau affect all six isoforms of tau. The insoluble tau aggregates that accumulate in the brain in patients with these mutations are seen on western blot to be composed of variable mixtures of 3R and 4R tau species, sometimes with a preponderance of 4R species (Murrell et al., 1999; Lippa et al., 2000; Pickering-Brown et al., 2000; Neumann et al., 2001; Kobayashi et al., 2003b; van Herpen et al., 2003), but on other occasions more 3R is present (Rizzini et al., 2000; Hogg et al., 2003). Curiously, the I260V tau mutation leads to the accumulation of only 4R tau species (Grover et al., 2003). However, the pattern of insoluble tau species in brain by western blot in cases with tau mutations has not, in some instances [for example, in the L266V mutation (see Hogg et al., 2003) and the G389R mutation (see Pickering-Brown et al., 2000; de Silva et al., 2003)], closely correlated with the pattern of tau immunostaining of Pick bodies where the robust presence of 4R tau on western blot has not been mirrored by a similar presence of 4R tau in Pick bodies upon immunohistochemistry. To explain this, it has been suggested (Hogg et al., 2003) that the failure to detect 4R tau species in Pick bodies by immunohistochemistry when insoluble 4R tau is detectable in brain homogenates may be due to the presence of ‘contaminating’ 4R tau species in western blots derived from 4R-immunopositive threads and glial cells rather than Pick bodies, the latter being composed solely of 3R tau species. Similar discrepancies between western blot and immunohistochemical findings which cannot be resolved in terms of differing cellular and anatomical compartmentalization of 3R and 4R tau species have been reported in sporadic FTD cases with Pick bodies [Delacourte et al., 1998 (but see Zhukareva et al., 2002; Taniguchi et al., 2004; for western blotting); Arai et al., 2001; Ikeda et al., 2002; de Silva et al., 2003 (but see Zhukareva et al., 2002; for immunohistochemistry)]. However, in this (present) Q336R case, although tau-immunopositive threads and astrocytes were (variably) present, the Pick bodies were still strongly reactive to both 3R and 4R tau antibodies. Unfortunately, no frozen brain tissue from this present case was available for study and so the precise balance of isoform composition of the insoluble tau within the tissue cannot be ascertained, though it may be presumed that, consistent with the other tau mutations associated with this form of pathology, at least some 4R insoluble tau isoforms would be present. Similarly, cases of sporadic FTD associated with Pick bodies are heterogeneous, with substantial quantities of 4R tau present within the Pick bodies (Zhukareva et al., 2002; Taniguchi et al., 2004). Hence, the Pick bodies in this present case of Q336R tau mutation differ, in terms of their tau isoform composition, from those in L266V tau mutation, implying (as in sporadic FTD) that it is not the balance of tau isoform species per se that determines the morphology of the tau pathology.

The reason for differences in tau isoform composition within Pick bodies between the various tau mutations remains unclear. Mutation position within tau per se is unlikely to determine this since the tau pathology associated with a tau mutation at the neighbouring codon (i.e. V337M mutation) (Poorkaj et al., 1998) is identical to that observed in Alzheimer’s disease with typical NFTs, not Pick bodies, composed of (approximately) equivalent amounts of 3R and 4R tau (Spillantini et al., 1996) being present. The phosphorylation state, as well as changes in amino acid composition, could influence the conformation of tau. Phosphorylation at Ser262 and Ser356 might be important in determining the morphology of the tau pathology present. As with sporadic forms of FTD with Pick-type histology (Probst et al., 1996; Delacourte et al., 1998; Arai et al., 2001; Ikeda et al., 2002; Zhukareva et al., 2002; Taniguchi et al., 2004), Pick bodies in FTDP-17 due to tau mutations are not (usually) immunolabelled by the phosphodependent antibody 12E8, which recognizes Ser262 and Ser356 when phosphorylated (Lippa et al., 2000; Rizzini et al., 2000; Neumann et al., 2001; Rosso et al., 2002; Hogg et al., 2003; Kobayashi et al., 2003a; van Herpen et al., 2003; Taniguchi et al., 2004). In contrast, this antibody strongly recognizes (most) NFTs in Alzheimer’s disease and FTDP-17 V337M tau mutation (Spillantini et al., 1996), suggesting that phosphorylation at Ser262 or Ser356, or both, may determine, at least partially, whether NFT- or Pick-type bodies are produced. However, the Pick-type bodies associated with L266V (Kobayashi et al., 2003b; but see Hogg et al., 2003) and G389R (Murrell et al., 1999) tau mutations, and sometimes those in sporadic Pick’s cases (Taniguchi et al., 2004), are 12E8 positive, showing that lack of phosphorylation at Ser262 or Ser356 is not an invariant or determining feature of Pick-type bodies in FTDP-17. Moreover, the NFT-like structures seen in neurons and glia in FTDP-17 tau mutations in and around exon 10 (Spillantini et al., 1996a; Pickering-Brown et al., 2002) are also 12E8 negative, reinforcing the point that a lack of phosphorylation at Ser262 or Ser356 is not mandatory for Pick body formation.
Therefore, it may be the conformational changes in tau that each tau mutation imposes (in conjunction with phosphorylation state) that are important in determining the structure of the tau aggregates, and the biochemical and pathological subtypes that develop, rather than the precise tau isoform composition. In its native state, tau is relatively unstructured and, consequently, the conformational impact of any mutation in tau will reflect the nature of the amino acid change as much as its location within the protein. In this way, each mutation could, in theory, act idiosyncratically, though, as seen above, the range of pathologies associated with missense tau mutations is relatively restricted, indicating that certain commonalities in the pattern of conformational change in tau by the different mutations do exist and it is these that may dictate the pathological outcome.

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