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Tau Pathology in a Family with Dementia and a P301L Mutation in Tau

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Abstract. Familial forms of frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) have recently been associated with coding region and intronic mutations in the tau gene. Here we report our findings on 2 affected siblings from a family with early-onset dementia, characterized by extensive tau pathology and a Pro to Leu mutation at codon 301 of tau. The proband, a 55-year-old woman, and her 63-year-old brother died after a progressive dementing illness clinically diagnosed as Alzheimer disease. Their mother, 2 sisters, maternal aunt and uncle, and several cousins were also affected. Autopsy in both cases revealed frontotemporal atrophy and degeneration of basal ganglia and substantia nigra. Sequencing of exon 10 of the tau gene revealed a C to T transition at codon 301, resulting in a Pro to Leu substitution. Widespread neuronal and glial inclusions, neuropil threads, and astrocytic plaques similar to those seen in corticobasal degeneration were labeled with a battery of antibodies to phosphorylation-dependent and phosphorylation-independent epitopes spanning the entire tau sequence. Isolated tau filaments had the morphology of narrow twisted ribbons. Sarkosyl-insoluble tau exhibited 2 major bands of 64 and 68 kDa and a minor 72 kDa band, similar to the pattern seen in a familial tauopathy associated with an intronic tau mutation. These pathological tau bands predominantly contained the subset of tau isoforms with 4 microtubule-binding repeats selectively affected by the P301L missense mutation. Our findings emphasize the phenotypic and genetic heterogeneity of tauopathies and highlight intriguing links between FTDP-17 and other neurodegenerative diseases.

Key Words: Corticobasal degeneration; Dementia; Frontotemporal dementia with parkinsonism; Mutation; Tau.

INTRODUCTION

Atypical dementias with frontal or frontotemporal degeneration and lacking typical Alzheimer disease (AD) neuropathology have defied ready classification. Appellations assigned to these disorders include dementia of frontal lobe type (1–4), frontotemporal dementia (5–6), progressive subcortical gliosis (7), primary progressive aphasia (8), dementia lacking distinctive histopathologic features (9), and lobar atrophy without Pick bodies (10).

From this large group has emerged a subset of families (11–15) designated as "frontotemporal dementia with parkinsonism linked to chromosome 17" (FTDP-17) by a consensus conference convened in 1996 in Ann Arbor (16). The term "multiple system tauopathy" was used by Spillantini and coworkers (17) to reflect the extensive tau deposition seen throughout multiple regions of gray and white matter in their family. Although the clinical and neuropathological features are heterogeneous, FTDP-17 is often characterized by neurobehavioral manifestations such as hyperorality or obsessive compulsive features, disturbance in cognitive function, and parkinsonism.

Linkage of these diseases to chromosome 17q21-22 has fueled the search for mutations in the tau gene, which lies within this region (13, 18–19). In adult human brain, 6 tau isoforms are produced from a single gene by alternative mRNA splicing (20). The isoforms differ by the presence or absence of inserts of 29 or 58 amino acids located in the amino-terminal half and a 31 amino acid repeat located in the carboxy-terminal half. Inclusion of the latter, which is encoded by exon 10 of tau (21–22), gives rise to the 3 tau isoforms with 4 microtubule (MT)-binding repeats.

Recently, 5 separate missense mutations and 4 separate intronic mutations (Fig. 1) have been described in families with FTDP-17 (23–27). Intronic mutations are located close to the splice-donor site of the intron following exon 10, where they disrupt a predicted stem-loop structure (24–26). This has been shown to lead to increased levels of the 3 tau isoforms with 4 MT-binding repeats and to reduced levels of the 3 isoforms with 3 MT-binding repeats (24–26). Overproduction of 4 MT-binding repeat tau isoforms may lead to an excess of tau over available binding sites on microtubules (25) resulting in the assembly of 4 MT binding-repeat isoforms of tau into wide twisted ribbon-like filaments (17). The known exonic mutations in tau are located in the MT-binding

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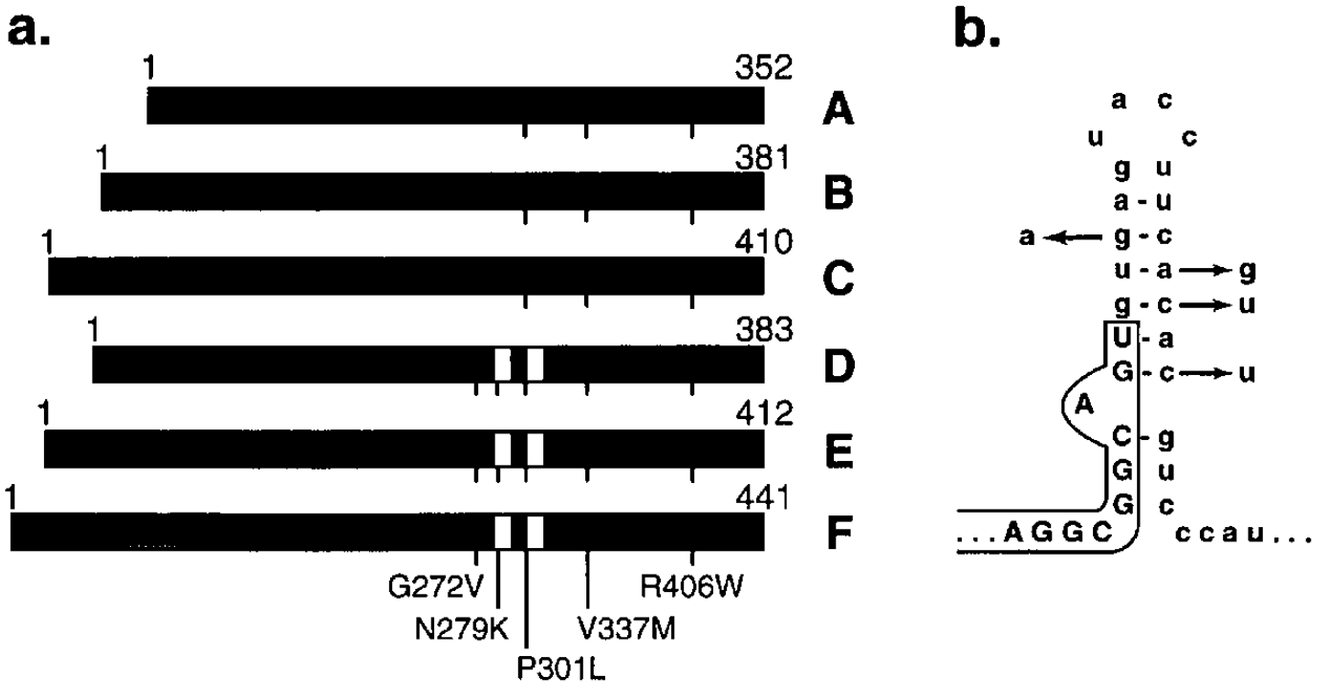

Fig. 1. Mutations in the tau gene in frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17). (**a**) Schematic diagram of the 6 tau isoforms (A–F) that are expressed in adult human brain. Alternatively spliced exons are shown in red (exon 2), green (exon 3), and yellow (exon 10), and black bars indicate the microtubule-binding repeats. Five missense mutations in the coding region are shown. G272V, V337M, and R406W affect all 6 tau isoforms, whereas N279K and P301L only affect tau isoforms with 4 microtubule-binding repeats. Amino acid numbering corresponds to the 441-amino-acid isoform of human brain tau. (**b**) Stem-loop in the pre-mRNA at the exon 10-5′ intron boundary. Four intronic mutations are indicated. Exon sequences are shown in capital and intron sequences in lower case letters.

repeat region or close to it, suggesting that they may interfere with the ability of tau to interact with microtubules (23–24, 26–27). The precise mechanisms by which these mutations lead to the assembly of tau into filaments and to neurodegeneration are only poorly understood.

In order to begin to understand genotype/phenotype correlations, it is essential that cases with known tau mutations be well characterized. Here we report neuropathological, genetic, and biochemical findings in 2 affected members from a previously unreported family with a Pro to Leu mutation at codon 301 of tau. This mutation has been described in other FTDP-17 families including a large Dutch kindred (family I of Heutink et al [28], 24, 29), 2 American kindreds (23, 24), 6 French families (27), and 4 families of French-Canadian descent (26). Thus, we also compare our findings with those reported in FTDP-17 families with this and other tau mutations.

MATERIALS AND METHODS

Pedigree

The family of the proband has been studied and, to the best of our knowledge, derives from the southeastern United States (Tennessee, Virginia, Florida, Georgia, and South Carolina). No known Dutch, French, or French Canadian ancestry has been identified. Six members of the family have been examined clinically, and autopsies have been performed on 2 affected members, the proband and her 63-year-old brother.

Neuropathology

Brain autopsies were performed on both the 55-year-old proband and her affected 63-year-old brother. Paraffin sections of formalin fixed brain tissue from multiple brain regions were stained with hematoxylin and eosin, thioflavine S, modified Bielschowsky, Sevier Munger, Bodian silver impregnation methods, and Woelcke-Heidenhain myelin preparation.

Immunohistochemistry was performed using antibodies to tau (rabbit polyclonal, Accurate Chemical and Scientific), ubiquitin (rabbit polyclonal, Dako), glial fibrillary acidic protein (GFAP; rabbit polyclonal, Dako), neurofilament (2F11, Dako), and β-amyloid (4G8, kindly provided by Dr. H. Wisniewski), linked with peroxidase using the avidin-biotin complex method (Vector) and developed with 3,3′-diaminobenzidine. Tau immunoreactivity was further characterized on sections of Case 1 (proband) using anti-tau antibodies that recognize phosphorylation-independent [Alz50 (30–31), 133 (32–33)] and phosphorylation-dependent [AT8 (34–35), PHF6 (36), PHF1 (37–38), T3P (38–40), and AP422 (41)] epitopes, spanning the whole tau sequence (20, 42) as shown in Figure 2.

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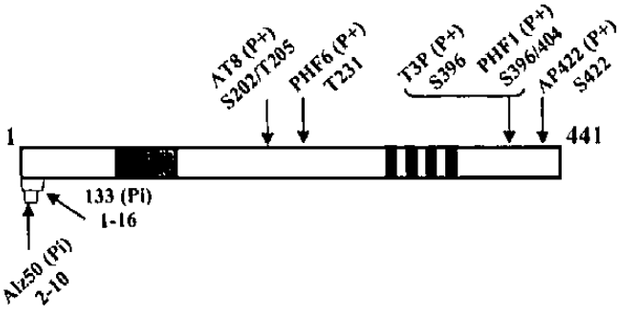

Fig. 2. Epitopes recognized by tau antibodies, which labeled tissue from Case 1, are indicated on this diagram of the tau molecule. Note that the epitopes recognized span the tau molecule and are phosphate-independent as well as phosphate-dependent. (Pi) = phosphate independent; (P+) = phosphate dependent; S = serine; T = threonine. The vertical bars on the right indicate the 4 C-terminal repeats; the black and shaded boxes on the left indicate the 2 N-terminal inserts.

Genetic Analysis

Informed consent was obtained from all participating family members. Blood or tissue samples were collected from 6 individuals. Genomic DNA from whole blood from 4 living family members was isolated using the protocol described by Madisen et al (43). Two DNA samples were obtained from paraffin-embedded sections (proband) and frozen brain tissue (case 2) as described (44). For PCR amplification of exon 10 of the tau gene, the primers 5′ CGAGCAAGCAGCGGGTCC 3′ and 5′ GTACGACTCACACCACTTCC 3′ from the intronic sequences surrounding exon 10 were used so that the entire exon sequence and the splice signals could be analyzed.

Standard amplification reactions were done with 20 ng/μl of genomic DNA. The amplified products were then gel purified. To generate single-stranded template for sequencing, asymmetric amplification was performed. A 1:50 ratio of the primers was used to amplify the samples for 40 cycles. The amplified products were then subjected to Qiaquick PCR purification spin columns (QIAGEN) which removed remaining primers and deoxynucleotides.

Standard dideoxynucleotide sequencing was performed using the U.S. Biochemicals Sequenase kit, ^{35}S-dATP (Amersham) and modified T7 DNA polymerase (Sequenase Version 2.0, U.S. Biochemicals/Amersham). The reactions were loaded and run on a 6% polyacrylamide/8M urea sequencing gel. Sequences were compared with those from 4 other family members, 125 controls, and from the National Center for Biotechnology Information Database. In addition, to more readily identify the presence of a missense mutation in this codon, MspI restriction enzyme digestion was performed on the exon 10 amplified product. The digestion products were then run on a 2% agarose gel and visualized by ethidium bromide staining.

Tau Extraction, Dephosphorylation, and Immunoblotting

Sarkosyl-insoluble tau was extracted from frontal cortex, temporal cortex, and hippocampal formation of the brother of the proband as described (32). For dephosphorylation, aliquots of sarkosyl-insoluble tau were treated with 6M guanidine hydrochloride and 2% 2-mercaptoethanol and incubated for 3 hours in the presence of 18U/ml *E. coli* alkaline phosphatase (type III-N, Sigma Fine Chemicals) at 67°C (32). Reconstituted samples of sarkosyl-insoluble tau were run on 10% SDS-PAGE and blotted onto Immobilon N. The blots were incubated overnight at 4°C with anti-tau antibody BR134 (20) and stained using the biotin-avidin Vectastain system (Vector Laboratories). Soluble tau was extracted using 2.5% perchloric acid, as described (45).

Electron Microscopy

Aliquots of sarkosyl-insoluble tau were placed onto carbon-coated 400-mesh grids and stained with 1% lithium phosphotungstate. Micrographs were recorded at a nominal magnification of ×40,000 on a Philips model EM208S electron microscope.

RESULTS

Clinical History

Case 1: The proband, a 55-year-old white woman, usually a fastidious person, first manifested problems with housekeeping. She experienced progressive decline in other activities of daily living, began losing checks, and had her driver's license revoked when she left the scene of an accident and forgot to appear for court dates. Neuropsychological evaluation 5 years prior to death revealed severe dysfunction of executive abilities associated with frontal lobe function, including perseverative responding, as well as impairment in naming, visuospatial function, and short-term memory, particularly memory acquisition. The patient was notably compliant and unaware of her deficits. Motor and sensory examination was unremarkable. The patient died in a nursing home in Tennessee approximately 7 years following the onset of her neurological disorder. Autopsy was limited to brain examination.

Case 2: Limited clinical information is available on the proband's brother. He was a 63-year-old man with a history of hypertension who experienced a stroke 6 years prior to death with mild hemiparesis and aphasia, but from which he made an almost complete recovery. Four years prior to death, he developed a gradually progressive dementia syndrome with confusion and short-term memory loss. He had 1 known episode of hallucinations. One year prior to death, his gait became slow and unsteady with "freezing spells," and he developed a resting tremor. He received anticholinesterase inhibitors without clear benefit. Several months prior to death, a CT of the head revealed marked frontal and, to a lesser extent, temporal lobe atrophy as well as dilatation of the lateral and third ventricles. He became comatose following respiratory difficulty and died.

Additional Family History

Pedigree analysis is shown in Figure 3, and some information is now available on 6 generations. Briefly, 14

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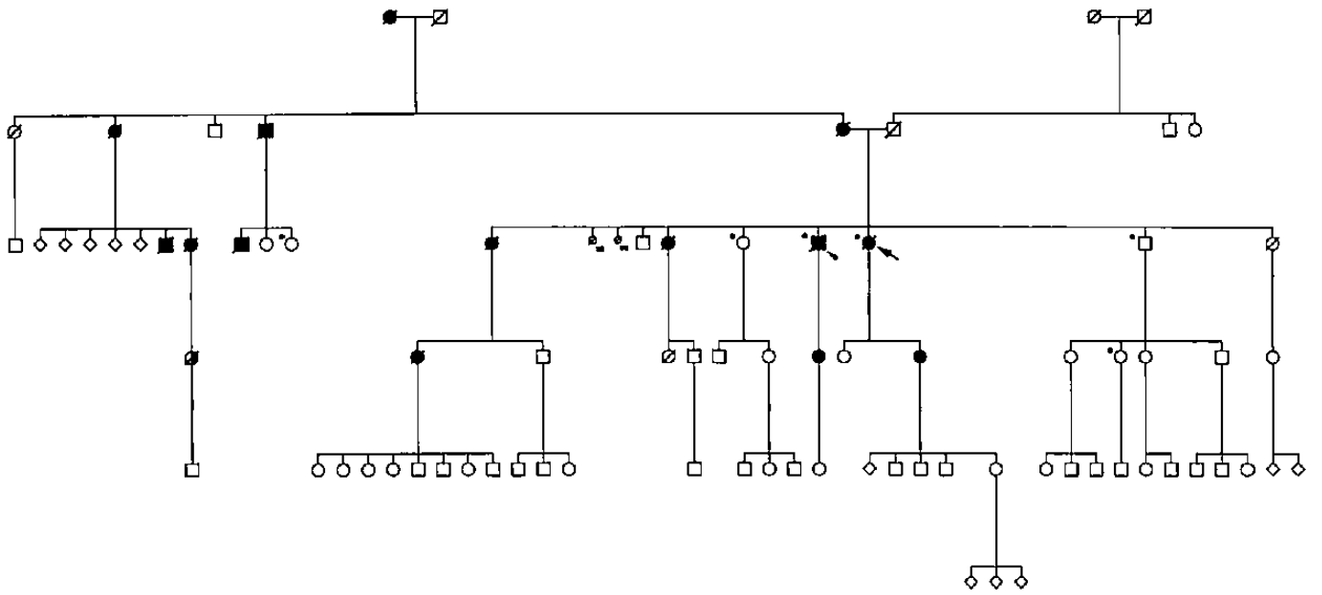

Fig. 3. Pedigree. The proband (arrow) a 55-year-old woman and her 63-year-old brother (arrowhead) are indicated. Half-filled symbols denote family member who may be affected but for whom adequate documentation is not available. A dot indicates that genetic analysis was performed. Note: The 2 gray-filled symbols denote family members (brother of the proband and his daughter) who evidenced cognitive impairment on neuropsychological testing; the daughter (niece of the proband) also reported obsessive-compulsive behavior. Yet repeated genetic analysis in both these individuals failed to reveal the P301L mutation.

family members within 4 generations were affected; 2 additional individuals may have been or are affected. Four of 8 siblings in the proband's generation (generation III) were affected and 3 or 4 individuals are affected in generation IV. The mother and 2 sisters of the proband (Case 1) died with memory disorders at ages 54, 57, and 60 years of age respectively, but were not autopsied. The sisters as well as a maternal uncle and aunt were clinically diagnosed as having AD. One of the sisters had experienced personality changes starting at about age 47 and developed enormous appetite with obesity and kleptomania. The patient's 63-year-old brother described above (Case 2) had memory problems as well as a history of a stroke with hemiparesis, aphasia, and hypertension. The proband's niece describes several family members as having obsessive compulsive behavior, including an affected sister of the proband who died without autopsy and herself; DNA analysis of this niece, however, failed to reveal the P301L mutation. Another 50-year-old niece died in a nursing home at age 50 years with a clinical diagnosis of AD.

Neuropathological Findings

Case 1 (Proband): The brain weighed 915 grams and exhibited marked atrophy of frontal, temporal, and parietal lobes, as seen in Figure 4a. The cerebral white matter and corpus callosum were also reduced in volume. The hippocampus and amygdala were unremarkable in size. The caudate nuclei were flattened with extensive dilatation of the lateral ventricles (Fig. 4b). The substantia nigra showed loss of pigment (Fig. 4c) and exhibited a bronze discoloration. The pons and medulla were unremarkable. The dentate nuclei appeared slightly bronze; the remainder of the cerebellum showed no abnormalities.

Microscopic examination revealed variable neuronal loss, gliosis, and focal vacuolization in atrophic regions of cortex as well as basal ganglia and substantia nigra. The most severe neuronal loss and gliosis were appreciated in the frontal cortex, middle and inferior temporal lobes, and parietal cortex. The cingulate and insular cortex exhibited moderate degeneration, and there was relative sparing of the occipital cortex. Thread-like neurofibrillary tangles staining with silver preparations and immunoreactive with tau antibodies were found in multiple cortical regions, as well as in white matter within neurons and glia (Figs. 5a–c, 6a, b). Neuropil threads were also present within the cortex. Achromatic or ballooned neurons were readily identified within the amygdala, entorhinal cortex, neocortex, and caudate nucleus and labelled with an antibody to neurofilament protein (Fig. 5e, f). Astrocytic plaques, most prominent in frontal cortex, were also observed (Fig. 6c, d). Beta-amyloid (Aβ) deposition or senile plaque formation was not appreciated.

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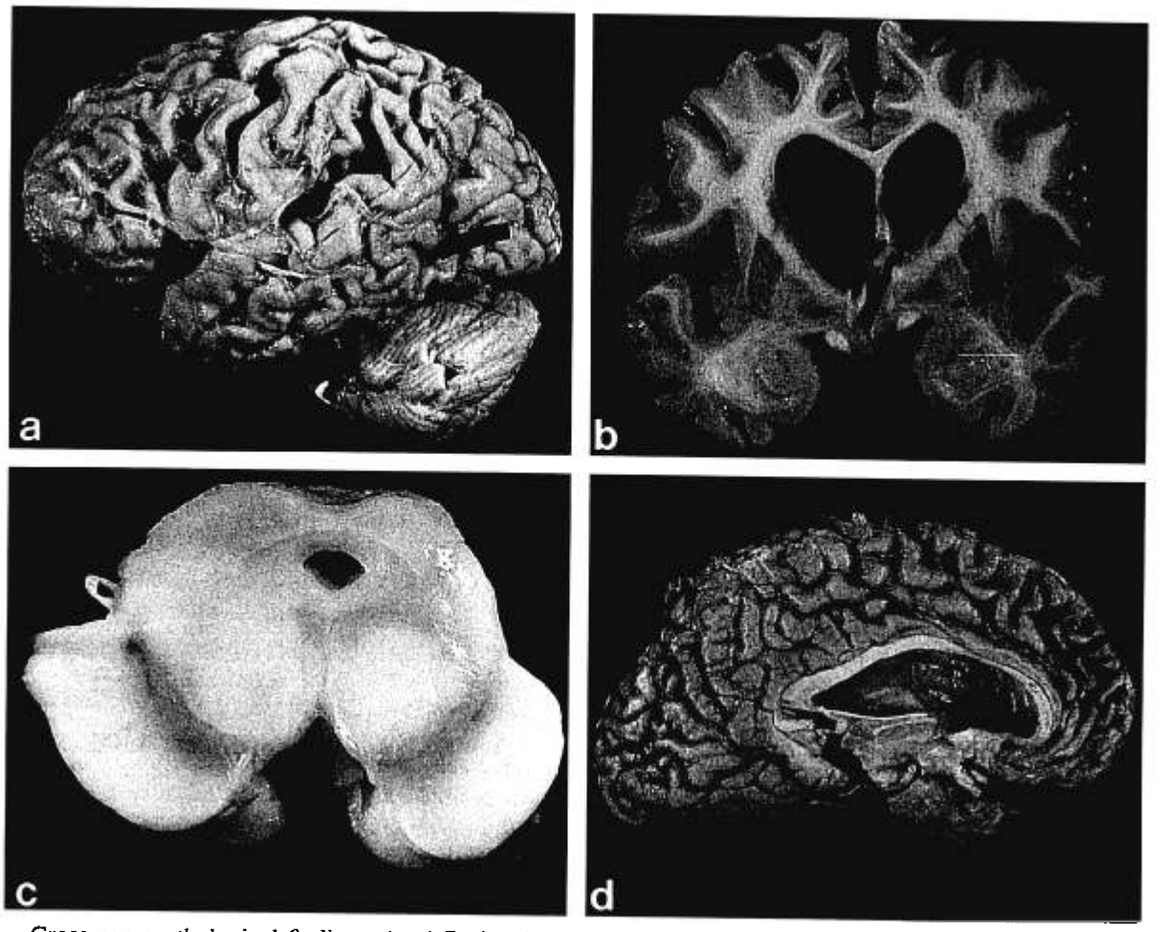

Fig. 4. Gross neuropathological findings. (**a–c**) Brain of the proband, a 55-year-old woman with dementia. (**a**) Lateral view demonstrating frontal, temporal, and parietal lobe atrophy. (**b**) Coronal section at the level of the amygdala showing cortical atrophy, attenuation of centrum semiovale and narrowing of corpus callosum, flattening of head of caudate nuclei and severe dilatation of the lateral ventricles. (**c**) Section of midbrain demonstrating pallor of bilateral substantia nigra. (**d**) Medial view of brain of the proband's 63-year-old brother demonstrating extensive frontal lobe atrophy.

Examination of the hippocampus with silver stains and tau immunohistochemistry revealed scattered tangle-like structures, most with a perinuclear orientation, within CA-1 of Ammon's horn. Tau-positive threads were common in the stratum oriens and alveus of the hippocampal formation. Moderate numbers of granule cells displayed tangle-like inclusions; labeling of the inner molecular layer by AT8, described by Spillantini and coworkers in a Dutch family with a P301L mutation (29), was not observed in the brain of the proband although such labeling was encountered in the brother (see below). Frequent thread-like tangles were seen in neurons throughout the entorhinal cortex and amygdala accompanied by glial inclusions in the cortex and white matter; these failed to label with ubiquitin antibody.

The caudate nucleus exhibited mild to moderate gliosis as well as frequent ballooned neurons. Abundant tau-positive thread-like tangles were seen in large neurons of striatum and within glia in white matter. The pallidum showed mild to moderate astrocytosis, striking mineralization of vessel walls, hemosiderin deposition and swollen axons or ovoids. Moderate neuronal loss and gliosis were observed within the nucleus basalis and many neurofibrillary tangles were seen in residual neurons on silver and tau immunohistochemical preparations. The thalamus was relatively spared with mild neuronal loss and moderate gliosis. Scattered neurofibrillary tangles were observed within periventricular gray matter. The subthalamic nucleus showed only mild gliosis but moderate numbers of neurofibrillary tangles, many thread-like, were observed within neurons.

The substantia nigra (Fig. 5d) exhibited striking neuronal loss with gliosis, swollen granular axons (ovoids), and iron deposition. Tau immunolabel revealed extensive reactivity within mesencephalic white matter, mainly involving glial inclusions; many neuronal inclusions were found scattered throughout the midbrain as well. The pons evidenced numerous intraneuronal tangles within

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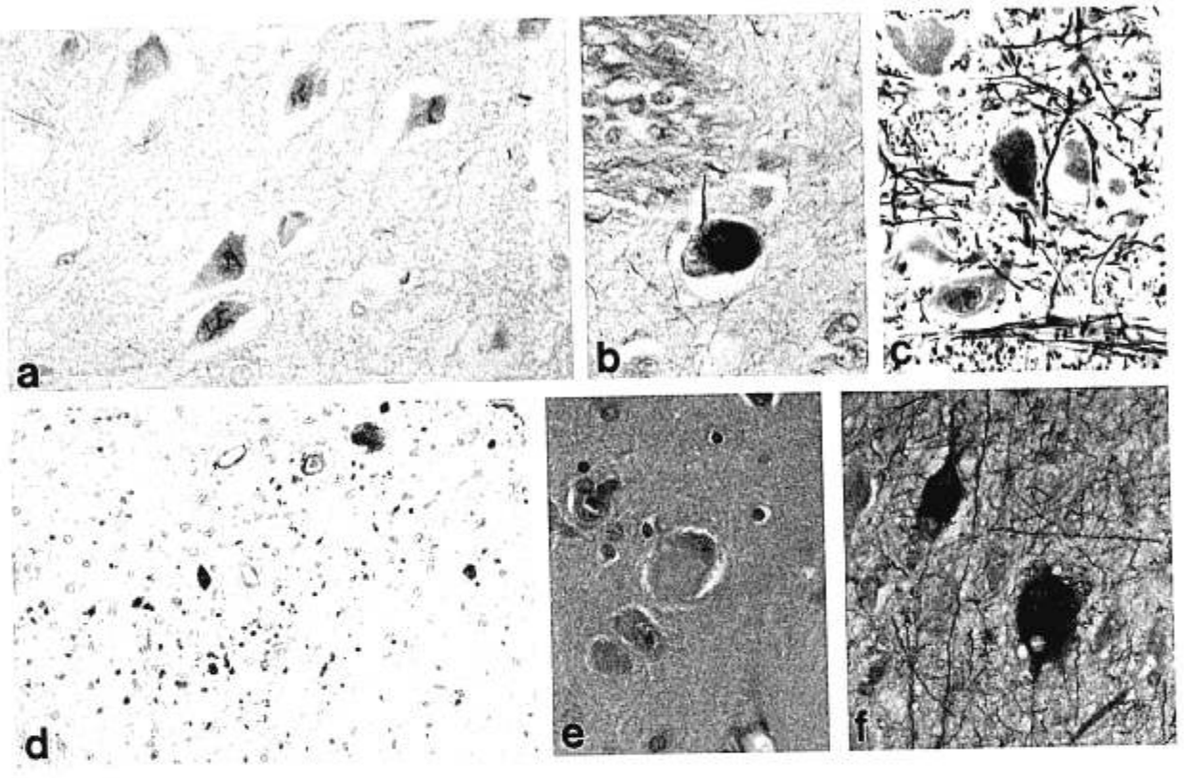

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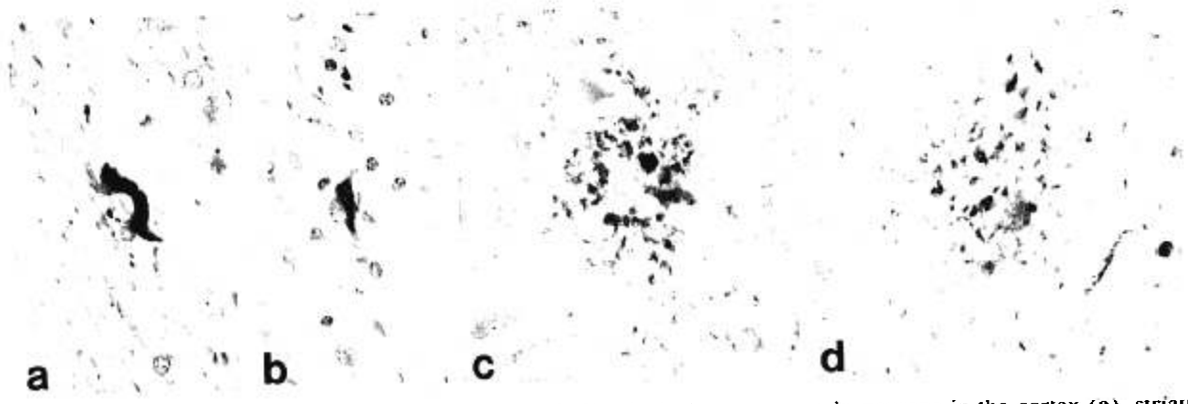

Fig. 5. Histopathological features. (**a–c**) Silver-positive tangle-like inclusions are seen in neurons in the cortex (**a**), striatum (**b**), and basis pontis (**c**) Sevier Munger ×188. (**d**) The substantia nigra exhibits marked astrocytosis, pigmentary incontinence, and siderosis. Hematoxylin and eosin ×63. (**e**) Ballooned cortical neuron. Hematoxylin and eosin ×125. (**f**) Ballooned neurons, 1 with cytoplasmic vacuole, are highlighted on neurofilament immunohistochemical preparation. Dako monoclonal neurofilament antibody, clone 2F11. ×125.

Fig. 6. Tau-positive inclusions. Tau-immunoreactive inclusions are seen in neurons (**a**) and glia (**b**) along with astrocytic plaque-like structures (**c** and **d**). (**a** and **c**) tau immunohistochemistry using rabbit polyclonal antibody from Accurate Chemical and Scientific. ×188; (**b** and **d**) AT8 ×188.

the basilar nuclei. Tau immunohistochemistry showed extensive positivity of neurons in the reticular formation and abundant immunoreactivity in pontine white matter. The medullary white matter showed similar immunoreactivity; scattered neurons within the inferior olivary nucleus showed thread-like neurofibrillary tangles.

The cerebellum exhibited mild siderosis in the region of the dentate nucleus, corresponding to the bronze appearance of this nucleus noted grossly. No grumose degeneration was observed. Moderate numbers of thread-like tangles were seen within dentate neurons. The Purkinje cells and internal granule cells were relatively well

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preserved. Some tau-positive glial inclusions were found within the cerebellar white matter.

As seen in Figure 6, the tangle-like inclusions in neurons and glia and astrocytic plaques were labeled positively with anti-tau antibodies. These structures were labelled with antibodies recognizing phosphorylation independent (Alz-50) and phosphorylation-dependent (AT8, PHF-1, T3P) epitopes and spanning the entire tau sequence. However they were labeled only weakly and less consistently with an antibody to ubiquitin.

Case 2: The brain weighed 1060 grams in the fresh state and exhibited moderate frontal and temporal lobe atrophy. The medial surface of the frontal lobes, including the anterior cingulate gyrus, was markedly atrophic (Fig. 4d). The left hemisphere was preserved for neuropathological examination. The lateral ventricle was dilated with flattening of the head of the caudate nucleus. The substantia nigra showed extensive pallor with slight bronze discoloration. Corresponding to the history of stroke 6 years prior to death, several old cystic infarcts were seen in the left basal ganglia and involving the internal capsule at the level of the thalamus. No definite abnormalities were appreciated in the brain stem or cerebellum.

Microscopic examination revealed degenerative changes similar to, but generally less severe than, those noted in the brain of the proband. Numerous tangle-like inclusions, neuropil threads, and occasional astrocytic plaques were seen on silver stain and tau immunohistochemistry within the cortex, along with rare ballooned neurons. Scattered Aβ-positive diffuse plaques were noted in the occipital cortex. The hippocampus revealed scattered thread-like silver and tau-positive inclusions within pyramidal cells of Ammon's horn, along with abundant neuropil threads. Cytoplasmic inclusions were identified in frequent granule cells. The inclusions varied in appearance from small and globular to more tangle-like; sparse to moderate numbers of AT8-positive threads, similar to the findings of Spillantini et al (29), were observed in the inner molecular layer of the hippocampal formation. Neuronal inclusions and neuropil threads were also prominent in the amygdala and entorhinal cortex. Striking neuronal loss and gliosis were seen in the substantia nigra along with siderosis; no Lewy bodies were observed.

Genetic Analysis

As seen in Figure 7, sequencing of exon 10 of the tau gene in the proband revealed a C to T transition at codon 301 resulting in a proline to leucine substitution. Sequence of exon 10 of the tau gene in the proband's brother also showed a C to T transition at codon 301 (data not shown). This change was not seen in 4 additional family members or in 125 controls from the Indiana DNA Bank. This nucleotide change eliminates an Msp I restriction site. Thus, when the amplified mutated exon 10 product

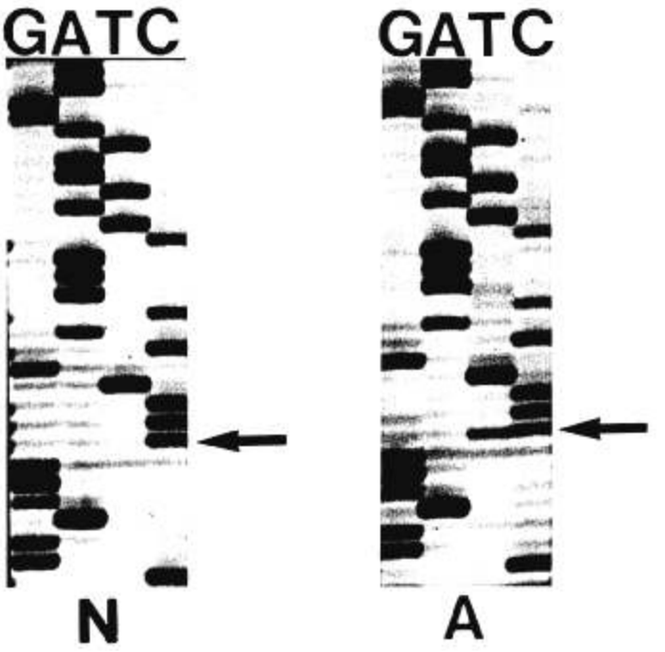

Fig. 7. Direct sequencing of exon 10 of the tau gene from a normal control (labelled N) and an affected (labelled A) member of the family. A C to T transition (marked by the arrow) in codon 301 results in a proline to leucine amino acid change in the tau protein.

was digested with Msp I, 3 bands of sizes 138, 82, and 222 basepairs (bP) are observed (Fig. 8). The 222 bp (uncut) fragment was not seen in 5 normal controls.

Immunoblotting of Sarkosyl-insoluble Tau

Sarkosyl-insoluble tau extracted from frontal and temporal cortices and hippocampal formation of the brother of the proband resolved on immunoblots into 2 major bands of 64 and 68 kDa and a minor band of 72 kDa (Fig. 9). Following alkaline phosphatase treatment, tau appeared as 3 major bands corresponding to the isoform with 4 MT-binding repeats and no amino-terminal insert (383 amino acid isoform) and the isoforms with 3 and 4 MT-binding repeats and the 29 amino acid amino-terminal insert (381 and 412 amino acid isoforms, respectively). The pattern of soluble tau was similar to that in control brain (data not shown).

Electron Microscopy

Electron microscopy of dispersed filament preparations from frontal and temporal cortices and hippocampal formation of the brother of the proband showed filaments structurally different from those found in AD. The filaments were narrow, irregularly twisted ribbons of width about 15 nm, with a crossover spacing greater than 130 nm (Fig. 10). By immunoelectron microscopy, the twisted ribbons were labelled by phosphorylation-dependent

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Fig. 8. Restriction enzyme digestion. The heterozygous C to T mutation eliminates a Msp I restriction site in 1 allele. When the amplified exon 10 product is digested with Msp I, 3 bands of sizes 138, 82, and 222 basepairs (bp) are observed. The 222bp (uncut) fragment is from the mutant allele and was not seen in normal controls. This 2% agarose gel shows these 3 bands when compared to the marker (Lane M) which is a 100bp ladder. Lane 1 is a normal control, lane 2 is the proband, lanes 3 and 4 contain DNA from brain and blood from the proband's brother, and lanes 5 and 6 are from a sister and cousin of the proband. Only the proband and her brother are affected and have the mutation.

and phosphorylation-independent anti-tau antibodies (data not shown).

DISCUSSION

The clinical-pathological features in our family with early-onset dementia and extensive tau pathology parallel findings in other FTDP-17 families, including those in which mutations in tau have been identified (46). Currently, these mutations fall into 3 groups, based on their locations in exon 10, the intron following exon 10, or the remainder of the tau sequence, chiefly the MT-binding repeat region (23–27).

The P301L mutation described here as well as a N279K missense mutation producing an amino acid change from asparagine to lysine in pallido-ponto-nigral degeneration (PPND) (26) belong to the first group. These mutations lie in exon 10, which is included in 4 MT-binding repeat, but not 3-repeat tau isoforms. It is likely that the interactions of mutated tau with microtubules are impaired, leading to an imbalance between functional 3-repeat and 4-repeat tau isoforms. This may in turn lead to the hyperphosphorylation of tau, and, perhaps through interactions with other factors (47), to assembly into sarkosyl-insoluble filaments. The latter are narrow, twisted, and ribbon-like, and consist predominantly of 4-repeat tau isoforms with only a small amount of the normally most abundant 3-repeat tau isoform. This indicates that the P301L mutation leads to the formation of tau filaments consisting predominantly of 4-repeat isoforms. The P301L mutation has also been found in Dutch family I (24), 2 American families (23–24), in 6 of 21 French FTDP families (27) and in 4 families of French Canadian descent (26, 23), suggesting that it is a relatively common mutation in FTDP-17 kindreds. In Dutch family I, an abundant neuronal and glial tau pathology has recently been described (29), with sarkosyl-insoluble tau and filament morphologies similar to those described in the present study. This indicates that the same mutation leads to similar biochemical and morphological changes in tau, irrespective of genetic background.

The neuropathological picture resulting from the P301L mutation is similar to that seen in familial multiple system tauopathy with presenile dementia (MSTD), an FTDP-17 with a G to A transition in the nucleotide adjacent to the splice-donor site of the intron following exon 10 (17, 25, 46). One difference is the absence of astrocytic plaques in familial MSTD. Another difference is that in familial MSTD, sarkosyl-insoluble tau consists

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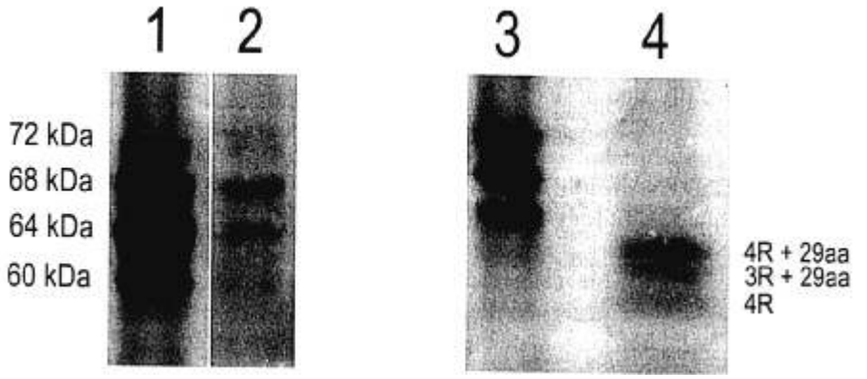

Fig. 9. Immunoblot of sarkosyl-insoluble tau from frontal cortex of patient with the P301L mutation in tau. Lanes: 1, Sarkosyl-insoluble tau from frontal cortex of AD patient; 2–4, Sarkosyl-insoluble tau from patient with the P301L mutation before (lanes 2 and 3) and after (lane 4) alkaline phosphatase treatment. Note the presence of pathological tau bands of 60, 64, 68 and 72 kDa in lane 1. The 60 kDa band is missing in lanes 2 and 3. Following alkaline phosphatase treatment, the pathological tau bands align with recombinant tau isoforms of 383 (4R), 381 (3R +29aa) and 412 (4R +29aa) amino acids. Immunoblotting was done using anti-tau antibody BR134.

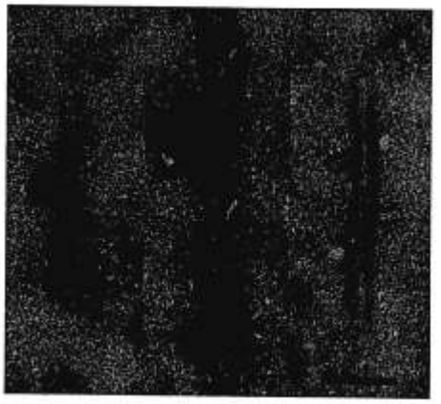

Fig. 10. Electron micrographs of sarkosyl-insoluble filaments from patient with the P301L mutation in tau. Three examples of the characteristic narrow twisted ribbon-like tau filaments are shown. Scale bar, 100 nm.

only of 4-repeat tau isoforms (17). The tau filaments are twisted ribbons (17) similar to, but wider than those found in families with the P301L mutation. Mutations in the intron following exon 10 lead to an overproduction of 4-repeat tau isoforms and reduced levels of 3-repeat isoforms (24, 25, 46). This may in turn result in an excess of 4-repeat tau isoforms over available binding sites on microtubules and be followed by the assembly of 4-repeat tau into filaments. It thus appears that mutations in exon 10 itself or in the intron following exon 10 lead to neuronal and glial tau pathologies with similar biochemical and morphological characteristics.

This contrasts with the third group of mutations. Where analyzed, the known missense mutations in exons 9 (G272V [24]), 12 (V337M [23]), and 13 (R406W [24]) of the tau gene lead to a predominantly neuronal pathology (29, 48–50). Seattle family A is characterized by a V337M mutation in the third MT-binding domain of tau, which leads to a filamentous tau pathology that is indistinguishable from that of AD in its biochemical and morphological characteristics (23, 49). The tau filaments are paired helical or straight filaments, which consist of all 6 tau isoforms. The same may be true of the R406W mutation in an Iowa family (24, 50). The G272V mutation in Dutch family II was associated with the formation of Pick-like bodies (29). However, the lack of frozen brain tissue has so far precluded a more detailed characterization.

In FTDP-17, there appears to be a remarkably direct correspondence between the positions of tau mutations and the biochemical and morphological characteristics of tau filaments. However, this is not always reflected at the clinical level, indicating that genetic background influences the manner in which mutations in tau lead to clinical manifestations. Thus, most of the affected members in our pedigree with the P301L mutation presented with memory loss resembling that seen in AD. By contrast, the most common presenting symptoms in Dutch family I were behavioral changes, particularly disinhibition (28). Interestingly, obsessive-compulsive behavior has been observed in some members of our pedigree, as well as Dutch family I (28) and other FTDP-17 families (11, 51), and in corticobasal degeneration (CBD) (52).

Similar heterogeneity has been observed in other, usually sporadic tauopathies, such as corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), Pick disease, and AD (53). In 6 of 11 cases of neuropathologically diagnosed CBD, we observed overlapping neuropathological features of 1 or more disorders, including AD, PSP, PD, and hippocampal sclerosis (54). All 6 patients had exhibited memory loss early in the course of their illness. There is also substantial pathologic overlap between CBD and Pick disease. In addition to focal and often asymmetric cortical degeneration, Pick disease and

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CBD show ballooned neurons, variable degeneration of the substantia nigra and basal ganglia, and tau-positive inclusions. Yet, Pick bodies, typically numerous in Pick disease, are absent or infrequently noted in CBD. Moreover, the hippocampus, a major site of pathology in Pick disease, is typically spared in CBD. Neurofibrillary tangles, glial inclusions, and tau-related pathology are more prominent in Pick disease than previously appreciated (55–56). Similarly, PSP exhibits remarkable neuropathological and clinical heterogeneity. We observed concomitant pathologic changes of AD, Parkinson disease (PD), or of both disorders in 12 of 20 PSP cases (57). Other coexisting pathologies included CBD (2 cases) and hippocampal sclerosis.

Interestingly, the electrophoretic banding pattern observed with sarkosyl-insoluble tau in our family resembled that seen in some of these sporadic tauopathies, namely PSP and CBD (57–62). In all 3 conditions, pathologic tau migrates as 2 major 64 and 68 kDa bands.

The discovery of FTDP-17 as a primary tauopathy has established that tau mutations can cause frontotemporal dementias (23–27). It is now important to establish the prevalence of tau mutations as genetic causes of neurodegenerative disease. Cases of CBD, PSP, and Pick disease should be investigated, particularly when there is a family history. It will be interesting to examine individual cases with overlapping pathologies, such as PSP and AD, as well as cases where different tauopathies affect different members of the same family. It is rapidly becoming clear that the tau gene is a major locus of inherited dementing disease.

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