Neuropathological Features of Frontotemporal Dementia and Parkinsonism Linked to Chromosome 17q21–22 (FTDP-17): Duke Family 1684

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Abstract. Frontotemporal dementia with parkinsonism (FTDP-17) is an autosomal dominant disorder that presents clinically with dementia, extrapyramidal signs, and behavioral disturbances in mid-life and progresses to death within 5 to 10 years. Pathologically, the disorder is characterized by variable neuronal loss and gliosis in the frontal and temporal lobes, limbic structures, and the midbrain. Autopsied individuals from some kindreds display abundant neurofibrillary change while others, including a single affected individual from Duke Family 1684, lack distinctive histological features and exhibit only mild neuronal loss and gliosis in limbic structures and subcortical nuclei when examined by routine silver stain. Recently, mutations in the microtubule associated protein tau have been shown to segregate with the disease in this family and in many other affected kindreds. In order to examine the distribution of tau deposits, we performed tau immunohistochemistry, immunoblotting, and immunoelectron microscopy of tau-containing filamentous. Immunohistochemistry revealed numerous tau deposits within glial cells and within neurons. Twisted ribbon-like filaments observed by immunoelectron microscopy were immuno-decorated with tau AT8 antibody. Sarkosyl-insoluble tau extracted from the hippocampus and cortex migrated as 2 major bands at 64 and 68 kilodaltons and a minor band at 72 kilodaltons, which after alkaline phosphate treatment appeared to contain mainly tau isoforms with 4 repeats. Furthermore, the ratio of soluble tau with 4 to 3 microtubule-binding repeats was increased. The role of tau mutations in this disorder is discussed in this paper.

Key Words: FTDP-17; Tau; Dementia; Parkinsonism.

INTRODUCTION

Frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) is a clinical pathological entity that can be distinguished from Alzheimer disease. The disorder is inherited in an autosomal dominant fashion and presents clinically with dementia, extrapyramidal signs, and/or psychiatric disturbances in mid-life. The disorder progresses to death within 5 to 10 years of onset. In October 1996, an International Consensus Conference was assembled in Ann Arbor, Michigan. The clinical, genetic, and neuropathologic data from 13 kindreds were collected and discussed (1).

Although these families have all been genetically linked to the same region on Chromosome 17, the disorder is clinically and pathologically heterogeneous. Some kindreds present predominantly with dementia (2, 5–8) or psychiatric symptoms (3, 4, 9), whereas others are dominated by parkinsonism (10, 11). Neuropsychologic testing, when performed, shows evidence of frontal lobe dysfunction (2, 11).

We report herein the clinical, neuropsychologic, genetic, and pathologic features of one of the linked kindreds and discuss similarities and differences in the clinical and pathologic phenotypes.

MATERIALS AND METHODS

The Family 1684 was ascertained through referral to the Joseph and Kathleen Bryan Alzheimer’s Disease Research Center (Bryan ADRC) at Duke University Medical Center. Procedures for the recruitment of subjects were approved by the Institutional Review Board. Permission for participation was obtained from next-of-kin for family members who were unable to provide informed consent. Records were obtained and examined on deceased family members and on family members who resided distant from the Bryan ADRC. A pedigree was constructed and is illustrated in Figure 1.

Evaluation of the affected individuals included review of medical history, a standard neurological examination, and neuropsychometric testing. Diagnoses of dementia were assigned according to NINCDS-ADRDA criteria. Frontal lobe dementia was ascertained according to the criteria of Bruni (12).

Autopsy pathology reports were available for review on 2 individuals, Subjects 27 and 29. Histological sections of brain tissue were reviewed from Subject 29, but unfortunately neither paraffin blocks nor fixed tissue were available for further study.

In Subject 37, a brain autopsy was performed. The left hemisphere was fixed in 10% neutral buffered formalin, and the right hemisphere was frozen for subsequent studies. Formalin fixed paraffin embedded tissue sections were prepared from the middle frontal, inferior parietal, and superior temporal cortex, and from the occipital lobe including Brodmann Areas 17 and 18. Sections of the entorhinal, frontal, parietal, superior temporal, occipital and insular cortex, the hippocampal formation at the level of the lateral geniculate nucleus, the thalamus including...
the dorsal medial and subthalamic nuclei, the basal ganglia midbrain, pons, medulla, and lateral cerebellum.

Routine histological stains included hematoxylin and eosin/luxol fast blue. Congo red, and microwave silver stain, which stain extracellular tangles and paired helical filaments very well but are relatively insensitive to intracellular neurofibrillary change. (Unpublished observations)

Phosphorylation dependent anti-tau monoclonal antibodies AT8, AT180, AT100, 12E8, and PHF1 were used as previously described (6, 7). AT8 recognizes tau phosphorylated at Ser 202 and Thr 205, PHF1 recognizes tau phosphorylated at Ser 396, and Ser 404 and AT180 recognizes tau phosphorylated at Thr 231 and Ser 235 (13). AT100 recognizes tau phosphorylated at Thr 212 and Ser 214 (14, 15) and 12E8 tau phosphorylated at Ser262 (16). Phosphorylation-independent anti-tau antisera BR133 and BR134 (17) were also used for immunoblotting.

Immunohistochemistry

Immunostain for ubiquitin (rabbit polyclonal prediluted Biomedica Foster City, CA) and tau (AT8 mouse monoclonal 1:20 Innogenetics, Belgium) was performed on all blocks. Briefly, sections were fixed in 10% neutral buffered formalin, embedded in paraffin, and cut at 8 microns. Tissue sections were incubated overnight at 4°C with the primary antibody in 50 mM Tris-HCl pH 7.4 containing 0.02% Tween 20 and 150 mM sodium chloride.

Sections were washed in 10 mM phosphate buffered saline (PBS) for 30 minutes and subsequently incubated for 2 hours at room temperature in biotinylated secondary antibody diluted 1:200 as previously described (6). Sections of amygdala, hippocampus, and superior temporal lobe were also immunostained with AT180, AT100 and 12E8, and PHF1.

**Tau Extraction and Analysis**

PHF-tau was extracted as described previously (17). Briefly, 1 gram of tissue was homogenized in 10 ml of mM Tris-HCl pH 7.4 containing 800 mM NaCl 1 mM EGTA and 10% sucrose. The homogenate was centrifuged at 14,000 rpm for 20 minutes and the supernatant was incubated with sarkosyl at a final concentration of 1% for 1 hour at room temperature. The solution was then spun at 40,000 rpm for 1 hour and the resulting pellet resuspended in 50 mM Tris-HCl pH 7.4 at 100 μl/g starting material. For dephosphorylation, aliquots of PHF-tau were treated with 3 M guanidine hydrochloride, dialyzed overnight at 4°C against 50 mM Tris-HCl pH 7.4 containing 1 mM phenyl-methyl sulfonyl fluoride, and incubated for 3 to 4 hours with E. coli alkaline phosphatase at 67°C as described (17). PHF tau samples were submitted to sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 10% polyacrylamide gels and blotted onto Immobilon-N (Millipore). Blots were incubated overnight at 4°C with primary antibodies and stained using the biotin-avidin (Vector Laboratories) stain system and 4-chloro-1-naphthol or 3,3-diaminobenzidine as substrate. Soluble tau was extracted using 2.5% perchloric acid as previously described (18).

**Electron Microscopy**

Aliquots of the PHF-tau preparations were placed on carbon coated 400-mesh grids, stained with 1% lithium phosphotungstate, and micrographs recorded at a magnification of 40,000× on a Philips Model EM208S microscope, as previously described (19). The primary anti-tau antibodies were used at 1:100 dilution. After reaction with the appropriate secondary antibody (BioCell), the grids were stained with 1% lithium phosphotungstate.
### TABLE

<table>
<thead>
<tr>
<th>Family</th>
<th>Mean age of onset</th>
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<td>55</td>
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<td>47</td>
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<td>4, 6, 31, 37</td>
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* Present report, previously unpublished.

### RESULTS

Published genetic studies of families with FTDP-17 are summarized in the Table. The pedigree of Family 1684 is shown in Figure 1. The family consists of 5 generations with 57 members. There are 14 affected family members in 3 generations. Linkage analysis has been previously reported (2). Multipoint analysis yielded a lod score of 5.53 at peak recombination fraction.

The proband, Individual 39 (Fig. 1) had onset of symptoms at age 52 years. Early complaints included depression, personality change, and multiple vague physical complaints including difficulty walking. Other family members described the proband as apathetic and at times explosively irritable. His behavior resulted in severe occupational and social disability. On neuropsychologic evaluation, the proband showed marked impairment in naming, visual perception, and executive function. However, the rapid forgetting and apraxia that are typical of Alzheimer disease were not observed (20). Magnetic resonance imaging was normal with no evidence of structural abnormalities. Positron emission tomography (PET) demonstrated reduced uptake in the anterior frontal and temporal lobes without evidence of the diffuse hypometabolism that is typical of Alzheimer disease (21). The disease has progressed inexorably since the presentation 5 years ago.

There is an anecdotal history of dementia and behavioral abnormalities in 4 affected individuals (Subjects 3, 5, 9, 11). There are clinical records of cognitive dysfunction in 5 individuals (Subjects 14, 15, 17, 33, 35), behavioral abnormalities in 5 (Subjects 24, 28, 30, 38, 40), and extrapyramidal abnormalities in Subject 35. The average age onset of disease in this kindred is 54.9 years, with a range of 45 to 63 years. The average disease duration in the 5 individuals from whom data is available was 9.2 years. Frontal lobe dysfunction with problems in judgment and problem solving, lack of insight, and poor social awareness were more prominent than complaints of impaired memory or temporal lobe function until late in the disease.

Limited pathology evaluation including review of slides from a 1982 autopsy of Subject 29 exhibited neuronal loss and gliosis in the temporal lobe, the oculomotor nucleus, and in the substantia nigra. Severe neuronal loss and gliosis was found in the globus pallidus. The substantia nigra displayed profound cell loss with numerous eosinophilic degenerating neurons similar in appearance to grumose change. However, Lewy bodies were not seen. No globus pallidus and subthalamic nucleus. Silver stains for plaques and tangles as well as Congo Red stain for amyloid were negative.

A detailed neuropathologic examination of Subject 37 was performed. There was mild cortical atrophy, which was severe in the temporal lobes. However there was marked ventricular dilation on coronal sectioning. The substantia nigra was pale. The subcortical structures showed only mild atrophy (Fig. 2). Mild neuronal loss and gliosis in the frontal and temporal lobes and mild cell loss in the cingulate gyrus was observed on microscopic examination. Moderate neuronal loss and gliosis in the amygdala and in the entorhinal cortex were present, while only mild neuronal loss was found in the hippocampal formation. There was profound cell loss in the substantia nigra with frequent eosinophilic degenerating neurons and severe cell loss in the locus ceruleus.

The thalamus and basal ganglia including the globus pallidus were, however, completely normal on routinely stained sections in this case. Silver stain for plaques and tangles and Congo Red stain for amyloid were completely negative in all of the examined sections.

Ubiquitin immunohistochemical preparations of middle frontal cortex revealed numerous dot-like structures that were similar in distribution to those seen in normal aging, but much more frequent (22). There were neuropil threads within the cortex. Similar dot-like structures and interfascicular threads were seen in the white matter. Interfascicular threads and structures resembling the silver grains of Braak were seen within the cortex and limbic structures (23). Rare glial flame-like structures were seen.

Immunostaining with tau AT8 antibody elicited a pattern similar to that seen with ubiquitin immunostaining, but much more intense. Within the parietal lobe, there was staining of neurons and glia especially within the deeper cortical layers. Stellate glial structures, broad, thread-like structures, and fluffy plaque-like structures were seen on tau immunostain. Within the white matter, interfascicular rods, dot-like structures, and corkscrew-type structures were seen. Within the superior temporal cortex, there was more intense staining. Small flame-like structures were seen within the extracellular matrix and...
presumed to represent neuronal or glial processes. Within the hippocampal formation, tau deposits were seen within the dentate neurons of the hippocampus, Sommer's sector neurons and glia, and occasionally within glia of zone CA2. Abundant deposits were seen within Layer 4 of the entorhinal cortex within both neurons and glia and appeared as dot-like structures within the neuropil. Within the amygdala, several patterns of tau deposition were noted: 1) dot-like structures within the neuropil, 2) diffuse label within normal appearing neurons and glia, and 3) rare flame-like tangles within glia. Within the substantia nigra, tau deposits were seen within neurons, glia, as dot-like structures, and very elongated interfascicular threads (Fig. 2). Tau deposits were also seen within the red nucleus, dorsal lateral, and ventral medial nucleus of the thalamus, subthalamus nucleus, pontine nuclei, nucleus ambiguus, and dorsal vagal nucleus.

Immunostaining with PHF1 also exhibited deposits within neurons and glial cells, appearing as dot-like structures and neuropil threads in a similar anatomical distribution. Overall, the staining was less intense than the pattern observed with the AT8 antibody. A similar, but less intense pattern was observed with antibody AT100 (data not shown).

Staining with AT180 antibody showed only a few dot-like structures and rare glial tangles. In the hippocampal formation and temporal cortex, antibody 12E8 recognized mainly granular structures like those in familial Multiple System Tauopathy with presenile Dementia (MSTD) (7).
Fig. 3. Immunoblots of soluble tau (A), sarkosyl-insoluble tau (B) and sarkosyl-insoluble tau after alkaline phosphatase treatment (C) from a patient from Duke Family 1684. A: Soluble tau from temporal cortex from a control subject (lane 1) and a patient from Duke Family 1684 (lane 2) detected using anti-tau antiserum BR134. Six tau isoforms are visible in both lanes 1 and 2. In the patient from Family 1684, unlike the control subject, tau isoforms with 4 repeats are more abundant than tau isoforms with 3 repeats. B: Sarkosyl-insoluble tau extracted from temporal cortex from an Alzheimer disease patient (lane 1) and a patient from Duke Family 1684 (lane 2) stained with anti tau antiserum BR133. The 60 kDa band present in Alzheimer disease (lane 1) is not present in the patient from Duke Family 1684 (lane 2). C: Sarkosyl-insoluble tau extracted from hippocampus from a Duke Family 1684 patient, before (lane 1) and after (lane 2) alkaline phosphatase treatment and stained with anti-tau antiserum BR133. Following alkaline phosphatase treatment the 64, 68, and 72 kDa bands appear as 2 major bands which correspond to tau isoforms with 4 repeats and no amino-terminal insert (4R) and with 4 repeats and 29 amino acid amino-terminal insert (4R+29aa).

Fig. 4. Electron micrographs of sarkosyl insoluble filaments. The filaments are flat ribbons with occasional twists (A, B) and label strongly with anti-tau antibody AT8 (C, D). Scale bar 100 nm.

Immunoblotting of sarkosyl-insoluble tau extracted from hippocampus and temporal cortex showed 2 major bands at 64 and 68 kilodaltons and a minor band at 72 kilodaltons (Fig. 3). Following alkaline phosphatase treatment these bands appeared to contain mainly tau isoforms with 4 repeats (Fig. 3). Immunoblots of soluble tau stained with both BR133 and BR134, which recognize the amino- and carboxy-terminus of tau respectively, showed an increase of tau with 4 repeats compared with tau with 3 repeats (Fig. 3).

Electron microscopy of sarkosyl-insoluble material from hippocampus showed small numbers of tau containing filaments, identified by labeling with anti-tau antibody AT8 (Fig. 4). The filaments had the form of flat ribbons, of width approximately 22 nm with occasional twists, where the ribbon is viewed edge on and shows a thickness of about 6 nm. In the face-on view, the appearance is often of 2 pairs of stain-excluding white lines with a central, more stain-penetrated dark line between them. The appearance is very similar to the filaments
seen in MSTD (7) and to the ribbons produced by un-

twisting of AD PHFs by treatment with 2% formic acid
(23). The filaments were decorated intensely by mono-

clonal antibody AT8, indicating the presence of phos-

phorylated tau.

DISCUSSION

The clinical, genetic, and neuropathological features of
13 kindreds with frontotemporal dementia and Parkin-

sonism linked to a critical region on Chromosome 17
were presented and discussed at an international consen-
sus conference in October 1996 (1). These kindreds are
clinically and pathologically somewhat heterogeneous,
but unifying pathological features include variable neu-

ronal loss and gliosis in the frontal and temporal cortices
with variable involvement of the limbic structures and of

the midbrain. This pattern of cell loss is similar to that
seen in Lobar Atrophy (26).

Tau deposits have also been observed in the chromo-
some 17 linked familial MSTD (7, 27, 28) Pallido Ponto

Nigral Degeneration (10, 11, 29, 30); Familial Progressive

Subcortical Gliosis (31, 32); Disinhibition Dementia Par-

kinsonism Amyotrophy Complex (DDPAC) (5, 9, 33);

Seattle family A (3, 4, 6, 28); HD-DD2 (34); an Australian

family (35); and Dutch families I and II (36, 37). Re-

cently, the genetic defect in several of these families has
been identified in the tau gene (38, 39, 40) (Table 1).

The “Irish” family or DDPAC (5, 8, 9, 33) was found-
ed by one affected individual who immigrated to the
United States. This family is characterized clinically by
marked disinhibition and elements of the Kluver-Bucy
syndrome, alcoholism, and aggressiveness with depres-
sion and psychotic features in some family members.

Neurofibrillary change and tau deposits throughout the
limbic system, similar to the findings in the presently
reported case have also been found in individuals from
the DDPAC family (5). C to T mutation in the splice
donor site of exon 10 of the tau gene has been reported
(39).

Pallido-Ponto-Nigral Degeneration (PPND) has also
been linked to the same region of Chromosome 17 and
exhibits similar features (10, 11, 29, 30). The PPND
family with nearly 300 members affected over 8 genera-

tions, exhibits an autosomal dominant disease characterized by

Parkinsonism with dystonia, dementia, pyramidal tract,
and frontal lobe dysfunction.

Familial MSTD presents clinically with short-term
memory deficits, dementia, and Parkinsonism (7, 27, 28).

The disease has affected 41 individuals over 7 genera-
tions. The mean age of onset is 49 years and it is inherited
in an autosomal dominant fashion. The pattern of tau de-
posits described and the tau containing filament structure
are similar to that seen in the presently reported Duke
Family 1684. Furthermore, Duke Family 1684, as in

MSTD, shows an increase of tau isoforms with 4 repeats
in soluble tau (28, 40). In MSTD, a nucleotide change G
to A, which segregates with the disease, has been found
in the intron containing the 5' splice donor site of exon
10 (40). A similar exon 10 splice site mutation segregates
with disease in Duke Family 1684.

In 3 Dutch families, the dementia has also been linked
to Chromosome 17 (36). The clinical presentation is
marked by personality changes, uninhibited and inap-
propriate behavior, emotional and social indifference,
and dementia. Although a previous study reported no tau de-
posits (36), recent data show that tau deposits are present
in both neurons and glial cells (37). In Dutch Family I
and II, missense mutations, which segregate with the
disease, have been described in the tau gene and they cor-
respond to P301L in exon 10 in Dutch Family I, and to
G272V in exon 9 in Dutch Family II (39).

An Australian pedigree linked to Chromosome 17 has
also been reported (35). This pedigree consists of 5 gen-
erations with 26 affected members with a similar disease
course. Detailed neuropathologic findings in this kindred
have not yet been reported, but tau deposits have been
described (35). In this family a mutation C to T in the
intron sequence corresponding to the 5' splice donor site
of exon 10 in the tau gene has been reported (39).

Familial Progressive Subcortical Gliosis (FPSG) Fam-

ily A (31, 32) is also linked to Chromosome 17. Affected
individuals present clinically with personality change,

disinhibition, and psychosis with dementia and depres-
sion developing at some point within the disease course.

In contrast, another Chromosome 17 linked family, Fa-
milial Presenile Dementia with Psychosis, also known as
Seattle Family A (4, 6, 32), presents clinically with pre-
dominantly psychotic or belligerent behavior which fre-
quently leads to a diagnosis of paranoid schizophrenia (3,
4). At autopsy, there is cell loss in the frontal and tem-

poral cortices. The amygdala and the entorhinal cortex
are atrophic. However, 3 of 4 described individuals ex-
hibited a normal substantia nigra (3, 4). Similar to other
reported cases, there were extensive neurofibrillary de-
posits containing tau protein (2, 6). These deposits are
mainly present in neurones and appear on immunoblots
as 3 major bands of 60, 64, and 68 kilodaltons and a
minor band of 72 kilodaltons. This pattern differed from
that seen in other Chromosome 17 linked families and is
more like the pattern seen in Alzheimer disease (6, 17,
28, 41).

In Seattle Family A, the genetic defect has been iden-
tified as a missense mutation V337M in exon 12 of the
tau gene (38). Thus the differing pathological pattern
seen in this family may be caused by tau mutation outside
exon 10, which may lead to predominantly neuronal tau
pathology and filaments consisting of all 6 tau isoforms.

(41)
FTDP-17 is a clinically and pathologically heterogeneous disorder caused by mutations in microtubule-associated protein tau (38–40). It is characterized pathologically by variable neuronal loss and gliosis with variable tau deposits within neurons and glia in the frontal and temporal cortices with variable involvement of the caudate, globus pallidus, and substantia nigra (1). There is also variable involvement of pontine nuclei and cranial nerve nuclei. Many of the glial deposits closely resemble structures that have been reported to be associated with corticobasal degeneration and progressive supranuclear palsy (24). The neuropathological features and biochemical characteristics of tau deposits in Duke Family 1684 are very similar to other families with tau gene mutations, particularly MSTD and DDPA (28, 39, 40; Spillantini, unpublished observations). A mutation in the tau gene is also present in Duke Family 1684. Correlation of tau pathology and the presence and type of mutations in the tau gene in FTDP-17 families will contribute to understanding tau function and significance of the neuropathological features present in the affected families.

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