INTRODUCTION

Frontotemporal dementias (FTDs) represent a group of neurodegenerative diseases clinically characterized by profound changes in personality and social behavior and are associated with a marked atrophy of the frontal and temporal cortex and often with additional subcortical degeneration (1).

The majority of FTD cases are sporadic, but in some families the disease occurs as an inherited disorder. In these families, FTD is inherited as an autosomal dominant trait and a subset of cases has been linked to a genetic defect on chromosome 17 (2). In many of these families, mutations have been described in the tau gene, which lies on chromosome 17q21.22 (3–6). The discovery of mutations in the tau gene showed that dysfunction in tau can cause neurodegeneration and dementia, and such families are described as having frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) (2). All of the FTDP-17 families in which tau mutations have been identified are characterized by abundant tau pathology in many areas of the brain. Furthermore, recent data suggest a positive association of the tau H1 haplotype with FTD (7).

However, tau gene mutations have been found in only about 10% to 15% of familial cases of FTD, and at least 2 families with FTD have been described that show linkage to chromosome 17 but do not have tau gene mutations or significant tau deposits (8, 9). In other families with FTD and motor neuron disease (MND), neuropathologically characterized by ubiquitin inclusions in the brain, the genetic defect has been linked to chromosome 9q21.22 (10). In a large Danish family with FTD, the genetic defect has been associated to chromosome 3 (11).

In this large and unique Danish pedigree, 22 individuals in 3 generations have been affected (11, 12). The average age of disease onset was 57 years and the average duration of the illness was 8 years (11, 12). The disease presented with a predominantly frontal lobe syndrome, but there was also evidence for temporal and parietal lobe dysfunction.

Based on the observation that the clinical and pathological features between FTD-3 and other forms of FTD appear to overlap, we attempted to investigate further the neuropathology present in FTD-3 patients and looked for characteristic lesions found in other FTD forms. Tau-immunoreactive neurons were present in the frontal cortex of the 3 patients examined. These tau-positive neurons were more abundant in the cortical region of the brain of patient II-12 compared to the same brain region of patients III-13 and III-17. Sarkosyl-insoluble tau extracted from the frontal cortex of case II-12 revealed the presence of tau filaments that appeared to contain 3 major bands of 60, 64, and 68 kDa on Western blot analysis. By electron microscopy it was shown that the tau deposits consisted of straight filaments and filaments similar to Alzheimer disease (AD) paired helical filaments (PHFs). Soluble tau was similar to controls. No β-amyloid deposits were found.
TAU PROTEIN IN FRONTOTEMPORAL DEMENTIA LINKED TO CHROMOSOME 3 (FTD-3)

MATERIALS AND METHODS

Patients

In the present study we have screened the frontal cortex from 3 affected Jutland individuals from the FTD-3 family for the presence of abnormal protein deposits. The cases used were II-12, III-13, and III-17 (12). The patients were examined by neurologist J.M. Brown and psychiatrist S. Gydesen and the detailed clinical, neuroimaging, and pathological features of all the family members assessed have been recently described (12).

Neuropathologically, these patients presented with global cortical atrophy more marked frontally, consistent with the brain imaging data and the macroscopical examination. Cortical degeneration was associated with neuronal loss and gliosis, most marked in anterior parts of the brain but spared the amygdala, hippocampus, and striatum. Milder changes were found in parietal, basal, and temporal cortex as previously described (12).

The FTD characteristic spongiosis in layer 2 of cortex was present. The duration of the disease was 21, 11, and 9 years, and the age at death was 76, 61, and 59 years for patients II-12, III-13, and III-17 respectively (12).

Immunohistochemistry

Thirty-μm sections of paraformaldehyde fixed tissue were cut from the frontal cortex of cases II-12 and III-13 and used free-floating for immunohistochemistry. No other brain region was available. For case III-17, formaldehyde-fixed, paraffin-embedded sections were provided also from frontal cortex.

In order to block endogenous peroxidases the sections were treated with methanol (20%)/hydrogen peroxide (1.5%) for 30 min prior to overnight incubation with primary antibodies at 4°C. The sections were washed in TBS-Tween 20 before incubation with secondary antibodies and staining was visualized using the biotin/avidin Vectastain system (Vector Laboratories, Burlingame, CA), as previously described (13).

The following primary antibodies were used: phosphorylation-dependent anti-tau monoclonal antibodies AT8 and AT100 (Innogenetics, Ghent, Belgium), 12E8 (kind gift of P. Seubert, and AD2 (kind gift of A. Delacourte). Antisera against α-synuclein, ubiquitin, superoxide dismutase, neurofilament, and neuroserpin C are specific for the carboxy-terminus of human neuroserpin (unpublished results).

All the above primary antibodies were used at 1/1,000 dilution except SMI 312, which was used at 1/200,000. Secondary antibodies (Vector Laboratories) were used at a dilution of 1/250 and staining was developed using 3,3 diaminobenzidine (DAB) (Vector Laboratories).

Sections stained with the anti-Aβ antiserum were pretreated with 90% formic acid for 5 min. Immunohistochemistry with 12E8 was carried out in parallel on AD sections, which were used as a control for the antibody staining. Gallyas staining was performed according to the standard protocol.

Tau Extraction, Dephosphorylation, and Immunoblotting

Fresh-frozen tissue was available from case II-12, but not from cases III-13 and III-17. For extraction of soluble tau, 200 mg of frontal cortex from II-12 and from 2 age-matched controls were homogenized in 0.5 ml 2.5% perchloric acid, as described (14). Dephosphorylation of soluble tau and immunoblotting were carried out as previously described (14). Sarkosyl-insoluble tau was extracted from the frontal cortex of II-12 as previously described (15). Insoluble tau extracted from frontal cortex of an AD patient was used as a control.

The phosphorylation-independent anti-tau antiserum BR133 (14), specific for the amino-terminus of tau, and the phosphorylation-dependent antibodies AT8 and 12E8 were used for immunoblotting. BR133 and AT8 were used at a dilution of 1/10,000 whereas 12E8 was diluted 1/5,000. Peroxidase-linked secondary antibodies (Dako) were used at a dilution of 1/2,500 and the immunoblots were visualized using the ECL system (Amersham, Uppsala, Sweden).

Electron Microscopy

Procedures for immuno-electron microscopy were as described (16). The primary antibody used was monoclonal antibody AT8 at 1/100 dilution. After incubation with the gold-labeled secondary antibody, the grids were stained with 1% lithium phosphotungstate. Micrographs were recorded at a nominal magnification of ×40,000 on a Philips EM208S microscope.

RESULTS

Immunohistochemistry

Tau-positive neuronal and glial inclusions were seen in the frontal cortex of patient II-12 using phosphorylation-dependent antibodies AT8, AT100, and AD2 (Fig. 1A–D). Tau-positive deposits were present in the same brain region of III-13 and III-17, although they were less abundant (Fig. 1E, F). In all cases, tau immunoreactivity in neurons was seen in the cell body and along neuronal processes. In these neuronal cell bodies, patches of tau accumulation were often observed (Fig. 1A, B). Coiled bodies were also found in some oligodendrocytes (Fig. 1C). Anti-tau antibody 12E8 did not stain any neurons or glial deposits, although it stained tangles in an AD brain used as control. Similarly, tau deposits were not clearly stained by Gallyas staining. No staining for β-amyloid was found and immunostaining for α-synuclein, ubiquitin, superoxide dismutase, neurofilament, and neuroserpin was normal.

Tau Extraction, Dephosphorylation, and Immunoblotting

Normal levels of 3-repeat and 4-repeat tau isoforms were present in soluble tau extracted from the frontal cortex of the II-12 brain (Fig. 2A). Sarkosyl-insoluble tau was extracted from the frontal cortex of II-12 and...
Fig. 1. Immunostaining of tissue sections from FTD-3 frontal cortex. Tau-positive neurons (A) and coiled body in an oligodendrocyte (C) immunolabeled with antibody AT8 in frontal cortex of patient II-12. Tau-positive cells were also stained with antibodies AD2 (B) and AT100 (D). Neurons were stained in cell bodies and processes. Neurons in frontal cortex of patients III-13 (E) and III-17 (F) stained with antibody AT8. Scale bars in A for A, E and F = 125 μm; in B for B, C and D = 70 μm.

it appeared as 3 major bands of 60, 64, and 68 kDa following immunoblotting with tau antiserum BR133 (Fig. 2B). The same pattern was seen with antibody AT8. In contrast, the phosphorylation-dependent anti-tau antibody 12E8, which labeled the PHF-tau pathology of the AD control, failed to label the sarkosyl-insoluble tau bands of II-12 (data not shown). Unfortunately, the small amount of tissue available did not allow for alkaline phosphatase treatment to be carried out on sarkosyl-insoluble tau.

Electron Microscopy of Tau Filaments

Tau-containing filaments in sarkosyl-insoluble material from frontal cortex were identified by electron microscopy of immunolabeled preparations (Fig. 3). Filaments were labeled by the phosphorylation dependent monoclonal
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Fig. 2. Immunoblotting of tau from FTD-3 brain. A: Soluble tau extracted from the frontal cortex of patient II-12 (lane 4). Comparison with soluble tau from frontal cortex of 2 control brains (lanes 2 and 3) and recombinant tau isoforms (lane 1). The position of the 6 tau isoforms is indicated by the lines. The tau bands were visualized using antiserum BR133. B: Sarkosyl-insoluble tau from the frontal cortex of patient II-12 immunoblotted with anti-tau antiserum BR133 (lane 2). Comparison with sarkosyl-insoluble tau from an AD brain (lane 1). The arrows indicate the positions of the 60, 64, 68, and 72 kDa tau bands.

Fig. 3. Electron micrographs of sarkosyl-insoluble filaments from FTD-3 brain. The filaments have been labeled with monoclonal antibody AT8. A, B: PHFs; C, D: Straight filaments. Scale bar: 100 nm.

antibody AT8. The filaments, which were mostly short fragments, had the appearance of Alzheimer-type PHFs (Fig. 3A, B) or straight filaments (Fig. 3C, D).

DISCUSSION

Frontotemporal dementia in this Danish family is inherited as an autosomal dominant trait with high penetrance, and the disease locus has been assigned to chromosome 3, although the candidate gene has not yet been identified (11, 12). Previous neuropathological studies (12, 17) had failed to identify any protein deposits, including neurofibrillary tangles, β-amyloid deposits, or Lewy bodies using silver staining and other routine techniques in this family. Furthermore, both papers report that the hippocampus and the amygdala are unaffected, while atrophy, mainly of the frontal cortex but also of temporal and parietal cortices, can be found. These neuropathological findings are in agreement with the diagnosis of frontotemporal dementia and not AD. In this study, we investigated the presence of common protein aggregates in the brain of patients from this family using immunohistochemical and biochemical techniques. We found tau protein deposits, and here for the first time we present the characterization of the tau pathology in the FTD-3 family.

The limited amount of deposits and the absence of ubiquitin inclusions differentiate FTD-3 from FTDP-17 and from FTD with MND, while the cellular distribution of tau deposits in neurons and glial cells, as well as their antigenic characteristics, differentiate tau deposits in FTD-3 from those in AD. Due to the small amount of tau deposits that we have observed, mainly in the younger patients, it is difficult to say that FTD-3 is a tauopathy, although it seems clear that the genetic defect present in FTD-3 can lead to tau accumulation. In our study of frontal cortex, the only brain region available to us (from 3 FTD-3 patients), we found tau pathology in neurons and some glial cells.

Small amounts of straight and PHF-like filaments, similar to those present in AD, were found in sarkosyl-insoluble preparation from frontal cortex of the older case. The filaments resolved by immunoblotting as sarkosyl-insoluble tau bands of 60, 64 and 68 kDa. This pattern of tau bands is similar to the characteristic bands of AD tau pathology, but it differs from AD tau because the tau bands, like the deposits, were not stained by antibody 12E8.

Unfortunately, the isoform composition of the pathological tau bands in the older case could not be determined directly because of the limited amount of frozen brain sample available. However, we compared the relative density of the 3 bands present in the sarkosyl-insoluble extract to those of the AD brain and obtained similar results. This, together with the filament morphology, suggests that as in AD, all 6 tau isoforms may be present in the sarkosyl-insoluble extract.
The 12E8 antibody failed to label the sarkosyl-insoluble protein in immunoblots and did not stain any cell in immunohistochemistry, suggesting that pathological tau in this family, unlike AD but similar to Pick disease (18, 19), is not phosphorylated at Ser262 and/or Ser 356.

Tau deposits were more abundant in case II-12 than in cases III-13 and III-17. This difference could be due either to the different duration of the disease in the 3 patients or to the different age at death. Patient II-12 was older and had a longer duration of the disease; however, the presence of concomitant AD can be excluded because no β-amyloid deposits were found in this and another study (12). Furthermore, some glial deposits not characteristic of AD were present, and antibody 12E8, which stains AD tangles, did not label tau deposits in the FTD-3 brains. Tau deposits were not stained by silver staining (11, 12), and we did not clearly detect them using Gal-lyas. This picture is reminiscent of some families with FTDP-17 where initially the presence of tau deposits was excluded using routine techniques and it was later observed by using anti-tau antibody AT8 (20).

In conclusion, the cellular distribution of the tau pathology, the absence of 12E8 staining, the absence of β-amyloid deposits and neuritic plaques, the preservation of the hippocampus and amygdala, and the clinical diagnosis led us to exclude that the tau pathology observed in the FTD-3 family could represent normal aging or initial AD.

Currently, 3 genetically distinct groups of familial FTD have been described. FTDP-17, which is caused by mutations in tau and has characteristic tau pathology, chromosome 9-linked FTD with MND with characteristic ubiquitin inclusions, and FTD-3, which shows mild tau pathology and lacks any other distinctive features. Some of the histological and clinical features found in FTDP-3 are also observed in familial FTD with MND and FTDP-17 cases, such as frontal cortex atrophy and spongiosis in layer 2 of cerebral cortex, suggesting the existence of common mechanisms underlying the development of pathology in genetically distinct FTD forms. Identification of the genes involved will provide new insights into the mechanisms of the neurodegenerative process, which will almost certainly involve interactions between the different genetic factors.

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REFERENCES


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