Familial multiple system tauopathy with presenile dementia: A disease with abundant neuronal and glial tau filaments

(microtubule-associated protein tau/familial disease)

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Communicated by Aaron Klug, Royal Society of London, London, United Kingdom, January 24, 1997 (received for review October 11, 1996)

ABSTRACT Neurofibrillary lesions made of hyperphosphorylated microtubule-associated protein tau constitute not only one of the defining neuropathological features of Alzheimer disease but also are present in a number of other neurodegenerative diseases with dementia. Here we describe a novel autosomal dominant disease named familial “multiple system tauopathy with presenile dementia,” which is characterized by abundant fibrillary deposits of tau protein in both neurons and glial cells. There are no detectable deposits of β -amyloid. The tau deposits are in the form of twisted filaments that differ in diameter and periodicity from the paired helical filaments of Alzheimer disease. They are stained by both phosphorylation-independent and -dependent anti-tau antibodies. Moreover, tau immunoreactivity coexists with heparan sulfate in affected nerve and glial cells. Tau protein extracted from filaments of familial multiple system tauopathy with presenile dementia shows a minor 72-kDa band and two major bands of 64 and 68 kDa that contain mainly hyperphosphorylated four-repeat tau isoforms of 383 and 412 amino acids.

Several neurodegenerative diseases are characterized by the presence of abundant neurofibrillary lesions within certain brain regions. These lesions consist of abnormal filaments that are made of microtubule-associated protein tau in a hyperphosphorylated state (1). By far the most common of these diseases is Alzheimer disease (AD), in which tau-positive deposits are found in neurofibrillary tangles (NFTs), neuropil threads (NTs), and neurites of plaques (1). Ultrastructurally, these deposits comprise paired helical filaments (PHFs) and straight filaments (SFs) (2). Biochemically, they contain all six adult brain tau isoforms in a hyperphosphorylated state that run as three major bands of 60, 64, and 68 kDa and a minor band of 72 kDa apparent molecular mass (3–9). Many hyperphosphorylated sites have been identified by mass spectrometry and peptide sequencing (10) and by phosphorylation-dependent anti-tau antibodies (1). The latter have greatly facilitated investigation of tau hyperphosphorylation in diseases other than AD. It thus has been shown that lesions made of hyperphosphorylated tau similar to those found in AD are present in Down syndrome (11), Niemann–Pick disease type C (12–14), Gerstmann–Sträussler–Scheinker (GSS) disease with tangles (15, 16), prion protein amyloid angiopathy (17), parkinsonism–dementia complex of Guam (18, 19), and familial presenile dementia with tangles (20, 21).

Tau-positive neurofibrillary lesions also are found in progressive supranuclear palsy (PSP) (22–24), corticobasal degeneration (CBD) (25–30), and Pick disease (31, 32). However, the morphology of tau-positive filaments in these diseases differs from that of PHFs and SFs (33). Moreover, the pattern of abnormal tau bands is different from that seen in AD. Thus, in PSP and CBD, the 64- and 68-kDa tau bands are observed, but the 60-kDa band is missing (22–24, 27, 30) whereas in Pick disease the 60- and 64-kDa tau bands are present (32), but the 68-kDa band is absent. PSP and CBD show tau pathology in glial cells as well as neurons (33, 34).

We describe here a familial disease with an autosomal dominant inheritance that is characterized by an abundant and widespread tau pathology in both nerve cells and glial cells in the absence of β -amyloid ($A\beta$) deposits. The clinical picture and the neuropathology indicate that this is a previously undescribed disorder, which we have named familial “multiple system tauopathy with presenile dementia” (MSTD). The clinical aspects of the disease and genetic linkage studies will be described elsewhere (see *Discussion*). In this paper, we present the novel molecular neuropathological characteristics. The tau-positive lesions stain *in situ* with a large number of phosphorylation-independent and -dependent anti-tau antibodies, as well as with a heparan sulfate antibody. By immunoelectron microscopy, the anti-tau antibodies decorate isolated filaments, which differ in morphology from PHFs and SFs. By immunoblotting, tau protein extracted from filament preparations is visualized as two major bands of 64 and 68 kDa and a minor band of 72 kDa, similar to the pattern observed in PSP and CBD (22–24, 27, 30). Upon dephosphorylation with alkaline phosphatase, two major tau bands are present that align with recombinant tau isoforms of 383 and 412 amino acids. This demonstrates that the filaments in familial MSTD consist mostly of two tau isoforms, each with four microtubule-binding repeats.

MATERIALS AND METHODS

Materials. Fresh-frozen tissue from hippocampus, temporal cortex, and frontal cortex of two patients with familial MSTD (aged 58 and 68 years) and of two AD patients (aged 65 and 78 years) was used for biochemical studies. Tissue blocks from cerebral cortex, hippocampus, subcortical nuclei, midbrain, brainstem, cerebellum, and spinal cord from three patients affected by familial MSTD (aged 58–68 years) and tissue blocks from cerebral cortex and hippocampus from three

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Abbreviations: AD, Alzheimer disease; NFTs, neurofibrillary tangles; NT, neuropil threads; PHFs, paired helical filaments; SF, straight filaments; PSP, progressive supranuclear palsy; CBD, corticobasal degeneration; MSTD, multiple system tauopathy with presenile dementia; $A\beta$, β -amyloid.

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patients with AD (aged 65–82 years) and two control subjects without neurological disorder (aged 53 and 70 years) were fixed in 4% formaldehyde and embedded in paraffin. Sections (10 μ m) were stained with hematoxylin and eosin, the Heidenhain–Woolcke method for myelin, the Bodian method for neurofibrils, and Congo red and thioflavin S for amyloid. For immunohistochemistry, sections were incubated with polyclonal and monoclonal antibodies raised against A β (antibody 2332; gift of V. M.-Y. Lee, University of Pennsylvania, Philadelphia), glial fibrillary acidic protein (BioGenex Laboratories, San Ramon, CA), heparan sulfate (antibody 10E4; Seikagaku America, Rockville, MD), and ubiquitin (Carpinteria, CA), as well as phosphorylation-dependent and -independent anti-tau antibodies. The phosphorylation-dependent anti-tau antibodies AT8, AT180, AT270, and AT100 (35) were obtained from E. Vanmechelen (Innogenetics, Ghent, Belgium); PHF1 (8) was obtained from P. Davies (Albert Einstein College of Medicine, New York) and 12E8 (36) was from P. Seubert (Athena Neurosciences, San Francisco). AT8 recognizes tau phosphorylated at Ser-202 and Thr-205 (in the numbering of the longest human brain tau isoform) (37), AT270 recognizes tau phosphorylated at Thr-181 (38), AT180 recognizes tau phosphorylated at Thr-231 and Ser-235 (38), PHF1 recognizes tau phosphorylated at Ser-396 and Ser-404 (39), and 12E8 recognizes tau phosphorylated at Ser-262 and/or Ser-356 (36). The phosphorylation-dependent epitope of AT100 is not known. For immunoblotting and immunohistochemistry, all mAbs were used at 1:500 whereas the phosphorylation-independent anti-tau sera BR133 (amino terminus) and BR134 (carboxyl terminus) (40) were used at 1:200; BR304 and BR189, which are specific for the amino-terminal 29- and 58-amino acid inserts of tau, were used at 1:500 (40). For immunohistochemistry, anti-A β serum 2332 (41) was used at 1:4000. The anti-ubiquitin antibody was used at 1:100 and 10E4 was used at 1:250. To investigate the presence of astrocytic plaques, 40- μ m vibratome sections were cut and incubated with anti-glial fibrillary acidic protein and anti-tau PHF1 and AT8 antibodies.

Immunohistochemistry. Tissue sections from familial MSTD, AD, and control brains were incubated overnight at 4°C with the primary antibody and were processed for single and double staining as described (42). When the anti-A β -antibody was used, tissue sections were preincubated for 5 min in 90% formic acid before incubation with the first antibody.

Tau Extraction, Dephosphorylation, and Immunoblotting. Sarkosyl-insoluble tau was extracted as described (7). For dephosphorylation, aliquots of sarkosyl-insoluble tau were treated with 7 M guanidine hydrochloride and 2% 2-mercaptoethanol and were incubated for 3 h with 13.5 units of *Escherichia coli* alkaline phosphatase at 67°C, as described (7). Sarkosyl-insoluble tau samples were run on 10% SDS/PAGE and blotted onto nitrocellulose. The blots were incubated overnight at 4°C with the primary antibody and stained using the biotin-avidin Vectastain (Vector Laboratories) system (7).

Electron Microscopy. Aliquots of sarkosyl-insoluble tau were placed on carbon-coated, 400-mesh grids and stained with 1% lithium phosphotungstate, and micrographs were recorded at a magnification of $\times 45000$ on a Philips Electron Optics (Eindhoven, The Netherlands) model EM301 electron microscope, as described (2). Procedures for immunoelectron microscopy were as described (2). The primary antibodies were used at 1:100 and, after reaction with the appropriate secondary antibody (Biocell Laboratories or Sigma), the grids were stained with 1% lithium phosphotungstate.

RESULTS

Clinical and Neuropathological Characteristics of Familial MSTD. MSTD has affected 41 individuals over 7 generations in a pedigree of 383 subjects, with a mean age of onset of 49

years and a range of ± 10 years. The disease is inherited in an autosomal dominant manner. Initial symptoms consist of disequilibrium and deficits in short term memory. Later, there is progressive cognitive decline, gait instability, bradykinesia, generalized axial and limb rigidity, superior gaze palsy, and dysphagia. Neurological examination has shown increased tendon reflexes and a positive Babinski sign. The last stage is characterized by a severe global cognitive decline, superior gaze palsy, and rigidity. In two patients, electromyographic evidence of peripheral denervation has been documented. Neuroimaging studies have revealed global cerebral atrophy with frontotemporal predominance in some individuals. The mean duration of illness is 11 years.

Neuropathological examination of eight patients who had died with familial MSTD showed nerve cell loss and tau-positive fibrillary lesions in cerebral cortex, hippocampal formation, substantia nigra, hypothalamus, periaqueductal gray, third and fourth cranial nerve nuclei, reticular nuclei, raphe neurons, and dorsal nucleus of the vagus nerve. In cerebellum, there was loss of Purkinje cells. In spinal cord, axonal swellings and a mild loss of nerve cells in conjunction with fibrillary lesions were observed in anterior horn and dorsal gray. Myelin staining revealed an extensive loss of nerve fibers in the propriospinal tract, as well as in ventral and lateral spinothalamic tracts and in the lateral vestibulospinal tract.

Immunohistochemistry. Tissue sections through a number of brain regions and spinal cord from three patients with familial MSTD were used. Phosphorylation-dependent and -independent anti-tau antibodies stained numerous deposits in temporal cortex, hippocampal formation, subcortical nuclei, brainstem, and spinal cord. Tau-positive deposits were present not only in nerve cells but also in a large number of glial cells, chiefly oligodendrocytes, as identified by their characteristic size and morphology. Some astrocytes, identified by glial fibrillary acidic protein staining, were also tau-immunoreactive; no astrocytic plaques were observed in either paraffin or vibratome-cut tissue sections. The lesions consisted of NFTs, NTs, and glialfibrillary tangles.

The phosphorylation-independent anti-tau antibodies BR133, BR304, BR189, and BR134 stained the above lesions. Phosphorylation-dependent anti-tau antibodies AT8, AT270, AT180, PHF1, and AT100 also stained NFTs, NTs, and glialfibrillary tangles, as well as long processes in white matter (Fig. 1). Antibody 12E8 stained similar structures, but the number of immunoreactive NFTs was smaller when compared with that observed with the other antibodies. In addition, 12E8 stained abundant granular deposits in both neurons and glial cells, particularly in temporal cortex (Fig. 1). Double-staining immunohistochemistry was then performed using AT8 and 12E8. In nerve cells, AT8 stained NFTs that were distinct from the 12E8-positive grains (data not shown). The latter also were stained by some phosphorylation-dependent anti-tau antibodies besides 12E8, such as AT180. The tau-positive lesions in neurons and glial cells also were stained by the anti-heparan sulfate antibody 10E4 (Fig. 2). No A β staining was observed in any of the tissues examined. Many of the tau-positive structures were ubiquitin-immunoreactive.

Electron Microscopy. Electron microscopy of dispersed filament preparations from familial MSTD brain showed filaments that were structurally distinct from the PHFs and SFs found in AD, PSP, familial presenile dementia with tangles, Down syndrome, and Pick disease (data not shown). Although filaments were not very abundant in the sarkosyl preparations examined, they could be identified by labeling with various antibodies against tau protein. The filaments appeared as slender twisted ribbons, ≈ 22 nm wide in their widest regions and ≈ 6 nm in their narrowest parts (Fig. 3). The twist along the filament was variable, with a spacing between crossovers of 140–300 nm. The filaments contain the whole tau molecule;

FIG. 1. Temporal cortex from a familial MSTD patient showing neuronal and glial cells stained with phosphorylation-dependent anti-tau antibodies. Neurons and glial cells in grey matter stained with antibodies AT8 (A), AT180 (D), and PHF1 (G). Antibody 12E8 stains granular deposits in neurons and glial cells in both grey (J) and white matter (K). In white matter AT8 (B and C), AT180 (E and F), and PHF1 (H and I) stain numerous glial cells with cytoplasmic inclusions (C, F, and I). (Magnifications: A, B, D, E, G, and H, $\times 140$; C, F, and I, $\times 448$; J, $\times 760$; and K, $\times 112$.)

they were decorated by anti-tau sera BR133 and BR134. Tau protein in familial MSTD filaments is phosphorylated at a number of sites; the filaments were homogeneously decorated by phosphorylation-dependent anti-tau antibodies PHF1, AT8, AT180, and AT100 (Fig. 3).

Immunoblotting of Sarkosyl-Insoluble Tau. Sarkosyl-insoluble tau was extracted from frontal cortex, temporal cortex, and hippocampal formation of two familial MSTD patients. After SDS/PAGE and immunoblotting with BR133 or BR134, two major tau bands of 64 and 68 kDa, as well as a minor band of 72 kDa, were observed. Similarly, the phosphorylation-dependent anti-tau antibodies AT270, AT8, AT180, 12E8, PHF1, and AT100 all stained the 64-, 68-, and 72-kDa bands (Fig. 4). Identical results were obtained using sarkosyl-insoluble tau extracted from separated gray and white matter. Antiserum BR304, which is specific for the first

29-amino acid insert in tau, recognized the 68- and 72-kDa bands whereas antiserum BR189, which is specific for the second 29-amino acid insert, only stained the 72-kDa band (Fig. 4). After alkaline phosphatase treatment, the sarkosyl-insoluble tau bands resolved into two major bands that aligned with recombinant tau isoforms of 383 and 412 amino acids (Fig. 5).

DISCUSSION

MSTD is a familial disease that is inherited in an autosomal dominant manner, with dementia, generalized bradykinesia and rigidity, as well as superior gaze palsy, as the major symptoms. Neuropathologically, many areas of the central nervous system, such as neocortex, subcortical nuclei, brainstem, and spinal cord, are affected. Tau-positive fibrillary pathology is

FIG. 2. Temporal cortex from a familial MSTD patient double-stained with the phosphorylation-dependent anti-tau antibody AT8 and the anti-heparan sulfate antibody 10E4. AT8 staining is shown in blue, and 10E4 staining is shown in brown (Nomarski optics). Note staining of some nerve cells and glial cells with 10E4 and double-labeling of nerve cells and glial cells with both AT8 and 10E4. (Bar = 23.5 μ m in A, 21 μ m in B, and 20 μ m in C.)

present in both neurons and glial cells, chiefly oligodendrocytes. These lesions are stained by all phosphorylation-dependent anti-tau antibodies studied, indicating that tau in familial MSTD is phosphorylated at a number of the same sites as in AD. One difference from AD is that, in familial MSTD, antibody 12E8 stained not only NFTs, NTs, and glialfibrillary tangles but also numerous granular deposits in neurons and glial cells. As in AD (43), nerve cells that were immunoreactive for hyperphosphorylated tau also stained with the heparan sulfate antibody 10E4. In familial MSTD, this was also the case for glial cells with tau pathology, suggesting that the coexistence of heparan sulfate and tau immunoreactivities may be a general phenomenon in neurodegenerative diseases with tau pathology. Recent findings suggest that an interaction between

FIG. 3. Immunoelectron microscopy of isolated filaments from familial MSTD brain. The top and bottom panels are unlabeled, and the others are immunogold decorated with phosphorylation-dependent (AT100, AT180, AT8, and PHF1) and phosphorylation-independent (BR133 and BR134) anti-tau antibodies. (Bar = 100 nm.)

tau protein and sulfated glycosaminoglycans may be a necessary event in tau filament formation (43).

By electron microscopy, filaments from familial MSTD brain appear as slender twisted ribbons that differ from PHFs of AD by having both a wider diameter and a different periodicity. They contain the whole tau molecule, which is phosphorylated at some of the same sites as in PHF-tau from AD brain. By SDS/PAGE, sarkosyl-insoluble tau from familial MSTD brain runs as two major bands of 64 and 68 kDa and a minor band of 72 kDa. After alkaline phosphatase treatment, these bands resolve into two major bands that align with recombinant tau isoforms of 383 and 412 amino acids, thus indicating that tau isoforms with four microtubule-binding

FIG. 4. Immunoblotting of sarkosyl-insoluble tau from AD and familial MSTD brains. The anti-tau antibodies used were the phosphorylation-independent antisera BR134, BR304, and BR189 and the phosphorylation-dependent antibodies PHF1, AT180, AT100, AT8, and 12E8. The arrows point to the sarkosyl-insoluble tau bands of 60, 64, and 68 kDa seen in AD.

FIG. 5. Immunoblots of sarkosyl-insoluble tau from familial MSTD brain before (lane 3) and after (lane 2) alkaline phosphatase treatment using antiserum BR133. Note that before alkaline phosphatase treatment, two major tau bands of 64 and 68 kDa and a minor band of 72 kDa are present; after alkaline phosphatase treatment, two major bands are visible that align with recombinant tau isoforms (lane 1) of 383 and 412 amino acids (isoforms D and E in schematic diagram).

repeats are selectively incorporated into filaments from familial MSTD brain.

Dominant inheritance of a presenile dementia, as well as the type and distribution of the tau-positive lesions, set familial MSTD apart from other neurodegenerative diseases. There is some overlap with PSP and CBD, in which tau-positive deposits also are present in glial cells (34). NFTs and glialfibrillary tangles are present in both PSP and familial MSTD although the tau pathology in familial MSTD is more widespread. In familial MSTD, neuronal and glial tau deposits are ubiquitin-immunoreactive, although some tangles have been reported to be ubiquitin-negative in PSP (44, 45). Furthermore, no tufted astrocytes (34) are present in familial MSTD. Tau deposits in PSP contain straight filaments that differ from the twisted filaments of familial MSTD (22). Filaments from both diseases contain two major tau bands of 64 and 68 kDa.

Of the diseases mentioned above, CBD would appear to be pathologically the closest to familial MSTD. Similar areas of the brain are affected in CBD and familial MSTD. However, in familial MSTD, astrocytic plaques, which are a hallmark of CBD (29), are not present. Moreover, fibrillary deposits of CBD contain straight and twisted filaments, the latter having a different diameter and periodicity when compared with twisted filaments from familial MSTD (27, 30). In CBD, as in familial MSTD, sarkosyl-insoluble tau runs as two major bands of 64 and 68 kDa and a minor band of 72 kDa (27, 30). The same pattern of staining with anti-tau antibodies is observed in both CBD and familial MSTD. However, unlike familial MSTD, in which we found mainly four repeat tau isoforms in sarkosyl-insoluble tau, the absence of these isoforms in sarkosyl-insoluble tau from CBD has been suggested (27, 30). Furthermore, antisera directed against exon 3 in tau have been reported not to stain tissue sections from CBD (28); by contrast, antiserum BR189, which recognizes the same exon (40), does stain some tau deposits in familial MSTD. Taken together, these differences indicate that familial MSTD is distinct from PSP and CBD. A genomic screen to determine the location of the gene causing familial MSTD is currently

being done. Preliminary data suggest that familial MSTD is linked to chromosome 17q21 (J.R.M. and B.G., unpublished data), the same region the tau gene localizes to (46). Sequencing of the tau exons in familial MSTD is in progress. Whether familial MSTD belongs to the group of frontotemporal dementias with parkinsonism linked to chromosome 17 (47) must await the identification of the gene defects responsible for these diseases.

Immunoblotting of sarkosyl-insoluble tau from familial MSTD before and after alkaline phosphatase treatment using isoform-specific anti-tau antibodies enables us to establish definitively the previously discussed (7) isoform composition of the PHF-tau bands. The most likely composition is as follows: the 60-kDa band contains the shortest tau isoform with three repeats and no amino-terminal inserts, the 64-kDa band contains the tau isoform with four repeats and no amino-terminal inserts and the isoform with three repeats and the 29-amino acid amino-terminal insert, and the 68-kDa band contains the tau isoform with four repeats and the 29-amino acid amino-terminal insert, as well as the isoform with three repeats and the 58-amino acid amino-terminal insert. The 72-kDa band contains the longest tau isoform with four repeats and the 58-amino acid amino-terminal insert. This assignment of tau isoforms is identical to that reported for recombinant tau phosphorylated with GSK3 β (6).

The present findings indicate that one can distinguish at least three types of tau pathology based on the pattern of tau bands and their isoform composition. The first includes AD (1), Down syndrome (10), familial presenile dementia with tangles (21), parkinsonism-dementia complex of Guam (19), Niemann-Pick disease type C (24), and Gerstmann-Sträussler-Scheinker disease with tangles (16). Three major pathological tau bands of 60, 64, and 68 kDa, as well as a minor band of 72 kDa, are observed. They consist of all six tau isoforms in a hyperphosphorylated state, and the corresponding filaments are PHFs or SFs. The second type of pathology is represented by Pick disease, in which only the 60- and 64-kDa tau bands are observed and in which the filaments are morphologically distinct from PHFs and SFs (32, 48). The third type of tau pathology includes PSP, CBD, and familial MSTD, in which the 64-, 68-, and 72-kDa tau bands are observed. Filaments from familial MSTD consist mostly of hyperphosphorylated, four-repeat tau isoforms of 383 and 412 amino acids. Filaments from familial MSTD are most similar to the twisted filaments of CBD although they have a different diameter and periodicity (27, 30). By contrast, SFs from PSP are distinct from the twisted filaments of familial MSTD and the SFs from CBD, AD, and Pick disease (22, 49). It remains to be established whether the tau bands of 64 and 68 kDa present in PSP and CBD comprise the same tau isoforms as the corresponding bands in familial MSTD. Previous studies have indicated some immunohistochemical differences between CBD and PSP in that antibodies against the second 29-amino acid amino-terminal insert of tau stain fibrillary pathology in PSP but not in CBD (28). As in PSP, we find staining with such antibodies in familial MSTD. One common characteristic of PSP, CBD, and familial MSTD is the presence of tau deposits in glial cells (32, 33). The origin of tau in glial cells is unclear because in normal human brain tau mRNA is found mainly in neurons. Future studies will investigate whether tau mRNA also is expressed in glial cells in some pathological situations.

In conclusion, MSTD is a novel familial dementing disorder that is characterized by abundant neuronal and glial tau protein deposits in many regions of the CNS. Ultrastructurally, these deposits are made of twisted filaments that differ from the tau filaments found in other neurodegenerative disorders. Biochemically, hyperphosphorylated tau bands of 64, 68, and 72 kDa are observed in familial MSTD, similar to the pattern found in PSP and CBD. These pathological tau bands consist mainly of two tau isoforms, each with four microtubule-

binding repeats. Thus, unlike AD, in which all six brain tau isoforms are found in PHFs and SFs, only some tau isoforms participate in the formation of filaments in familial MST. The mechanisms underlying the incorporation of only some tau isoforms into filaments remain unknown. It could result from an abnormal level of expression of certain tau isoforms, a differential distribution of tau isoforms in some cell types, the selective action of protein kinases and/or protein phosphatases on individual tau isoforms, or the preferential interaction between some tau isoforms and other factors, such as sulfated glycosaminoglycans (43). The unraveling of these mechanisms will reveal what separates and what unites the various tau diseases.

We thank Francine Epperson, Brenda Dupree, and Rosemarie Funkhouser for technical assistance. Part of this work was supported by Public Health Service Grants NS29822 and P30 AG10133 (B.G.).

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