Specific Pathological Tau Protein Variants Characterize Pick's Disease

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Abstract. Pick's disease (PiD) is characterized by a pan-laminar frontotemporal cortical atrophy, widespread degeneration of the white matter, chromatolytic neurons, and Pick bodies (PB). Microtubule-associated Tau proteins are the main cytoskeletal components modified during these neurodegenerative changes. In the present study, pathological alterations of Tau proteins were investigated in the brains of five PiD cases at both neuropathological and biochemical levels, using the monoclonal antibody AD2 which recognizes a phosphorylation-dependent Tau epitope and strongly labeled PB. A large number of cortical and subcortical regions were studied on frozen materials. Tau proteins were analyzed on mono- and two-dimensional gel electrophoreses using a quantitative western blot approach. In all specimens, a 55 and 64 kDa Tau doublet was observed in limbic, frontal, and temporal cortices as well as in striatum and substantia nigra. In contrast, Alzheimer's disease (AD) brains are characterized by the presence of the 55, 64, and 69 kDa Tau triplet whereas the 64 and 69 kDa doublet is more typical of progressive supranuclear palsy and corticobasal degeneration. Thus, the 55 and 64 kDa doublet appears to be specific to PiD, less acidic than AD Tau proteins, and well correlated with the presence of PB.

Key Words: Chromatolytic neurons; Pathological Tau proteins; Phosphorylation; Pick bodies; Pick's disease; Two-dimensional gel electrophoresis.

INTRODUCTION

Pick's disease (PiD) is a rare type of presenile dementia, neuropathologically confined to the prerolandic frontal convexity, orbitofrontal gyri, anterior temporal, and occasionally parietal lobes (1), despite the frequently documented more diffuse forebrain atrophy with striatal involvement at post-mortem examination (2–4). The salient lesions are chromatolytic neurons and PB (5). The white matter usually displays a severe Wallerian degeneration in most affected areas (2). However, lobar atrophy without PB has also been reported (6, 7). PB were recently associated with progressive supranuclear palsy (PSP) (8) and have been observed together with variable densities of neurofibrillary tangles (NFT) and senile plaques in senile Alzheimer's disease (AD) (9).

The accumulation of Tau proteins in intraneuronal inclusions bodies was reported in several neurodegenerative disorders, including PiD (10–17). Ultrastructural studies have revealed paired helical filaments (PHF) in AD (16, 18), Down syndrome (19), Amyotrophic lateral sclerosis/Parkinsonism-dementia complex of Guam (ALS/PDC) (20) and Dementia Pugilistica (21, 22). They have shown straight filaments in PSP (23, 24), corticobasal degeneration (CBD) (25), and postencephalitic parkinsonism (PEP) (26). In ALS/PDC and PEP, however, straight filaments may be mixed with PHF (20, 27). A review of previous biochemical analyses emphasized the notion that abnormal polymerized Tau proteins are assembled into filamentous structures (28). Their variant profile may be related to the ratio of specific Tau isoforms assembled as filaments, and results in their pathological intraneuronal accumulation. Thus, a triplet of abnormally phosphorylated Tau proteins (Tau 55, 64, 69) polymerizes into PHF in AD and ALS/PDC (28–31), while straight filaments are made of Tau protein doublet of 64 and 69 kDa in PSP and CBD (25, 32, 33). Despite the heterogeneous structure and distribution of the neuronal inclusions, the strong correlation previously reported between the presence of pathological Tau protein isoforms in association cortex and intellectual impairment suggests that they are reliable biochemical markers of yet—incompletely understood fundamental pathophysiological processes.

Ultrastructurally, PB consist mostly of bundles of disorganized straight filaments, which may be mixed with coiled fibrils of 157 nm periodicity and immunoreactive with antisera to Tau proteins (17, 34). We report that by western blot techniques, their Tau component profile was characterized as a 55 and 64 kDa doublet, which specifically differed from that of PHF and straight fibers neuronal inclusions seen in neurodegenerative disorders other than PiD. Their molecular specificity was further supported by two-dimensional gel electrophoresis.

MATERIALS AND METHODS

Case Descriptions

All autopsy materials were obtained from both the IMAGE project brain bank and the Canadian Brain Tissue Bank. Post-mortem delays were from 10 to 50 h. All cases presented with some clinical signs of PiD including frontal disinhibition, in-
## Table 1

Quantitative Analysis of Neuropathological Lesions and Tau Markers in Pick's Disease.

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Distribution of senile plaques (SP), neurofibrillary tangles (NFT) in Pick's disease cases 1 and 2, and Pick bodies in cases 1–5. Scores for pathological Tau proteins (τ) in the cerebral cortex of all 5 Pick's disease cases are compared to typical Alzheimer (AD) and progressive supranuclear palsy (PSP) patients. Results represent the number of Pick bodies/mm², NFT/mm² or SP/mm² using modified Bielschowsky silver staining (MB) and AD antibody labeling (AD2) (35). Scores for τ proteins (55, 64 in Pick's disease; 64, 69 in PSP and 55, 54, and 69 in AD) are also given in %. 100% is an arbitrary value given to the AD2 immunoreactivity obtained in Brodmann area 22 from a typical AD patient. CA1: Ammon horn 1; Entorhinal Ctx: entorhinal cortex; N.b. Meynert: Nucleus basalis of Meynert; Ext. Pallidum: external Pallidum; Int. Pallidum: interior Pallidum; Thalamus doro-med: thalamus doro-median; Thalamus ventro-lat: thalamus ventro-lateral; Sub. Thal. Nucleus: sub-thalamic nucleus; Loc. Coeruleus: locus coeruleus; Oliv. Nuc.: Olivary complex.

appropriate judgement, and progressive language impoverishment. Cognitive deficits and memory impairment were also observed in some patients and may explain why they were diagnosed as AD. Neuropathologically, lobar atrophy predominated in frontal and anterior temporal lobes. PB were always present. After both clinical and neuropathological examination, PD diagnosis was established for all of the cases.

**Case 1** was a woman that manifested increasingly severe mood disturbances and self-neglect. At onset, a gradually worsening memory deficit was reported. Further observations revealed aggressivity, temporospatial disorientation, and lack of insight. A global confusion with marked dyspraxia appeared, and she then became incontinent. Later, she was in a vegetative state. After a 16-year clinical course, she died at age 61. The working clinical diagnosis was that of possible AD. At autopsy, the brain weighed 988 g. The hippocampal formation was severely affected by degenerative changes. Little was left of the dentate gyrus, and only few PB were observed (Table 1). PB were present in the temporal lobe. In frontal, parietal and cingulate cortices, few NFT were present in residual isocortical pyramidal neurons, and there were very rare senile plaques (Table 1). A moderate gliosis was present. The substantia nigra showed degeneration without neuronal loss, NFT or Lewy bodies.
Case 2 was a woman who died at age 70 after a 14-year clinical course. Onset occurred with memory deficits, followed by dysphasia and agnosia. Later, severe disorientation, apraxia, and marked loss of autonomy were noted. Aggressiveness and severe mood swings were also noted. Two years before death, pallidum was observed, as well as alexia, which coincided with the onset of recurrent myoclonic jerks. The working clinical diagnosis was that of probable AD. Brain atrophy (1058 g) was moderate. Particularly, the hippocampal formation was much less atrophic than in other cases. Very high densities of PB were observed throughout the dentate gyrus and Ammon's horn pyramidal layer (Table 1). Few intracellular NFT were also present. Both limbic regions and neocortex also displayed multifocal hypertrophic gliosis. There was minimal degeneration without neuronal loss in the substantia nigra and locus coeruleus. Senile plaques were rare (Table 1).

Case 3 was a woman who demonstrated onset of gradual memory loss. One year later, she became confused, unable to carry a conversation, with marked loss of words and verbal fluidity. A CAT scan showed moderate cortical atrophy. She died at age 65 with severe dementia after an 11-year clinical course from disseminated ovarian carcinoma. At autopsy, the brain weighed 880 g. Marked atrophy predominated in fronto-temporal lobes bilaterally, with moderate involvement of parietal and occipital regions. Severe neuronal loss with many PB were seen most in limbic lobes, which were not as extensive in parietal lobes. The mesial thalamus was moderately atrophied. There was slight atrophy of the substantia nigra and locus coeruleus. Amyloid deposition was not observed.

Case 4 was a woman who died at age 63. Early in the clinical course, all characteristic clinical signs of Pick's disease were observed including mood disturbances, loss of social awareness, verbal stereotypes, and oral activities. She died after 3 years of a dramatic and rapid development of the disease. At autopsy, the brain weighed 950 g. A severe fronto-temporal atrophy was observed. PB were numerous in layers II and III of all frontal and temporal areas. The hippocampal formation including dentate gyrus and entorhinal cortex was severely atrophic and PB were abundant. A moderate amount of PB was observed in the basal ganglia and thalamus midline. No NFT or amyloid deposits were observed. An important gliosis was observed in the fronto-temporal and hippocampal areas.

Case 5 is a 72-year-old man who began a cognitive decline, which for the next 3 years was mostly focused on deficits of short and long term memory. He then became dysphasic and markedly apraxic. He died after 7 years of relentless cognitive deterioration. At autopsy, the brain weighed 984 g. Lobar atrophy predominated in frontal and anterior temporal lobes. At microscopy, limbic lobes were mostly degenerated, with mild extension into the striatum. Severe neuronal loss and gliosis were present in frontal, temporal, and limbic regions, PB were also abundant and widespread in these areas. Sparse NFT were also observed. Amyloid deposition was not observed.

Preparation and Sampling of Materials

In cases 1 and 2, 1.0 cm slices of one cerebral hemisphere were snap-frozen in isopentane on dry ice, placed in sealed bags and stored at −80°C. Slices of brainstem and cerebellum were also prepared. The contralateral hemispheres were fixed whole in buffered 10% formalin. Snap-freezing of left and right hemispheres was alternated from case to case. The sampling of formalin-fixed histological sections included representative blocks of cortical and subcortical areas (Table 1). All blocks were cut and sections from each block stained with Hematoxylin Phloxin Saffron, modified Bielschowsky and alkaline Congo Red preparations. Lesions were counted on a Zeiss Axiohist microscope with an ocular graticule on modified Bielschowsky stains from each paraffin block sampled, with a 10× objective for PB and senile plaques/mm², and with a 20× objective for NFT/mm², according to previously described morphometric methods (35).

The surface areas used for counting with both objectives were pre-calibrated on a H.O.M.E computerized system interfaced with a Zeiss Axiohist microscope. The microscopic fields used for counting in each area were selected after careful screening of all cortical layers for the most severe foci of degenerative lesions. Adjacent sections were also used for AD2 immunohistochemistry of PB and NFT (Table 1). AD2 immunocytochemistry was carried out according to the indirect peroxidase-antiperoxidase method of Sternberger at a dilution of 0.8 µg/ml.

The frozen specimens were dissected by chiseling 1.0–1.5 × 1.5–2.0 cm large pieces of cortex and brain tissues. In cases 1, 2, and 5, twenty-four regions were sampled, weighed, homogenized and solubilized in SDS buffer. The following regions were analyzed: Brodmann's areas 4, 7, 9, 10, 11, 17, 18, 22, 23, 39, amygdala, head of caudate nucleus and putamen, internal segment of the globus pallidus, substantia innominata, dorsal nucleus of thalamus, and brainstem including substantia nigra, superior colliculus, pontine nuclei, inferior olive, dentate nucleus, and cerebellar cortex. Further biochemical studies were also done on exhaustive samples from all regions of frontal cortex. In case 3, ten regions were dissected for biochemistry. In case 4, only 4 samples of frontal, temporal, and parietal association cortex were analyzed (see Table 1). In cases 3, 4 and 5, the opposite half-brain was then fixed in 10% formalin. Classical neuropathological examination was then performed.

For biochemical analyses, tissues extracted from an AD brain were used as an internal standard for 55, 64, 69 kDa Tau proteins. The samples had been harvested from a patient who met all DSM IIIIR criteria for clinical dementia and the NINCDS-ADRDA criteria for definite AD (36). The patient died at 65 after a rapid cognitive decline. Other AD brains were also investigated, as described in reference 30. Other tissues from a previously described case of PSP were used as an internal positive standard for 64 and 69 kDa Tau protein variants (33).

Antibodies

AD2, a monoclonal antibody directed against a phosphorylation site located on pathological Tau proteins, was chosen for the present study because of its specificity and sensitivity (31, 37). The epitope is located on the C-terminal part of the Tau molecule (31), at phosphorylated Serines 396 and 404 (Bade-Scherrer et al, in press). Indeed, AD2 is able to detect pathological Tau proteins in crude SDS brain homogenates (31). It was also used to visualize the epitopes by light and electron microscopy (38). In all cases, AD2 immunohistochemistry was performed as described in 31. Other antibodies were also used, including GF5, a monoclonal antibody raised against human GFAP (39) and Tau-
I (Boehringer Mannheim GmbH, Germany) which is directed against a dephosphorylated site at region 192-204, numbering according to the longest Tau isoform (40). Tau-1 was used at the concentration suggested by the manufacturer.

**Immunoblots**

Brain tissue samples were heat-treated with the Laemmli sample buffer at a ratio of 1 g per 10 ml (30, 41). Ten µl of each homogenate were then loaded on SDS-PAGE (10 to 20% slab gel). Proteins were transferred onto Nitrocellulose membranes (0.45 µm pore size, Schleicher & Schuell) for 90 min (current: 0.8 mA per square centimeter) using an LKB Multiphor II Nova Blot according to the manufacturer's instructions. Blocking was carried out with TBS containing 5% (w/v) dry milk and 0.05% (w/v) Tween 20. The blotted proteins were incubated with the protein A-purified monoclonal antibody AD2 at a concentration of 0.3 µg/ml for 2 hours at room temperature. They were then revealed with horseradish peroxidase-labeled sheep anti-mouse immunoglobulins (Diagnostics Pasteur) and detected with the ECL western blotting system (Amersham).

**Two-dimensional Gel Analyses**

Two-dimensional (2D) gels were performed according to a modified O'Farrell's method (42). The first dimension gel contained 9.5 M urea, 2% Triton X100, 5% Pharmalytes (Pharmacia) with 4% of pH 3 to 10 and 1% of pH 4 to 6.5. Twenty µl of brain homogenate in Laemmli sample buffer were heat-treated for 5 min at 100°C and centrifuged at 10,000 g for 10 min at 4°C. Thirty-five µl of a solution containing 8 M urea and 4% Triton X-100 were added to the supernatant. Samples were electrophoresed for 20 h at 500 V, then focused for 30 min at 700 V on 10 cm long, 3 mm diameter capillary tubes. The first dimensional gels were then equilibrated for 5 min in 5 ml equilibrium buffer containing 10% glycerol, 0.5% DTT, 3% SDS and 0.25 M Tris at pH 6.8. The second SDS-PAGE dimension and western blot analysis were performed as described for immunoblotting.

pH gradient was calibrated with the Carbamylate® calibration kit for 2-D electrophoresis (Pharmacia). Five µl of carbamylated creatine phosphokinase (CPK) was added to the brain homogenate, as recommended by Pharmacia. The 2D gel was stained with Coomassie Blue after 2D electrophoresis in order to check the resolution and the linearity of the pH gradient. GFAP immunostaining was also used for calibration after AD2 staining. Tau-1 was either used as the first antibody, the blot then stripped according to Amersham ECL western blotting system, then stained with AD2, or the process was reversed (i.e. AD2 staining first, stripping followed by Tau-1 staining).

**Biochemical Quantitative Analysis**

Intensity of AD2 immunoreactivity was quantified by densitometry as previously described (30, 33). The intensity was then scored using arbitrary values relative to the signal obtained with the AD case, which was given a value of 100%.

**RESULTS**

**Immunohistopathological Results**

Immunohistochemical analysis using AD2 monoclonal antibody fully confirmed that all of the investigated Pick cases exhibited PB in frontal, temporal and limbic regions consistent with PiD diagnosis (Table 1; Fig. 1 A–C). It should be noted that AD2 immunocytochemistry always revealed larger densities of PB (>40%) than classical neuropathological examination using modified Bielschowsky staining (Table 1). PB regional and laminar distributions were similar in all cases and consistent with
data from previous studies (1, 3, 8, 9). PB were mainly observed in supragranular and pyramidal layers (II, III) in frontal and temporal areas (Fig. 1A). The hippocampal formation contained high densities of PB, sometimes restricted to the granule cell layer of the dentate gyrus (Fig. 1B). At high magnification, the AD2 antibody revealed the round, target-like shape typical of PB (Fig. 1C), compared to the more irregular, flame-shaped and dispersed filamentous framework of NFT.

Case 1 was the least affected patient. Low numbers of PB were found in the hippocampal formation. In fronto-temporal areas, PB densities were moderate. In occipital cortex, PB were absent. With the exception of caudate nuclei, only rare PB were found in subcortical structures. Sparse NFT were observed in all of the investigated areas but absent in the subcortical structures (except a few in substantia nigra and locus caeruleus) (Table 1).

Case 2 displayed moderate amounts of PB. The hippocampal formation contained higher PB densities than neocortical areas. Fronto-temporal regions had moderate PB counts whereas other neocortical structures including parietal cortex did not display any PB. Among subcortical areas, caudate nuclei and putamen displayed PB. Rare NFT were also found (Table 1).

Case 3 was particularly affected in limbic areas. He displayed severe amounts of PB in the hippocampal formation whereas moderate densities were found in frontal and parietal cortex. Sparse NFT were also found.

Case 4 was the most affected case. For instance, PB counts were higher than 500 PB/mm² in the granule layer of the dentate gyrus. The hippocampal formation displayed very high amounts of PB. Fronto-temporal areas contained high PB densities (Table 1). Basal ganglia also demonstrated moderate PB amounts. With the exception of the entorhinal cortex, none of the investigated areas displayed NFT.

Case 5 was also severely affected with abundant PB densities in limbic structures including amygdala, hippocampus, and entorhinal cortex. High numbers of PB were also found in fronto-temporal areas. NFT were sparse and restricted to the hippocampal formation, locus caeruleus and substantia nigra.

Biochemical Results: Immunoblot Analysis

Several brain areas sampled from five PiD brains were studied, using a previously described quantitative western blot approach (30, 33). In all cases, except case 4, more than 20 different brain areas were investigated. Immunodetection of pathological Tau proteins was performed with AD2. Results were similar for all PiD cases (Fig. 2). Two major bands were detected, with a molecular weight (MW) of 55 and 64 kDa. They were also detected in parietal areas from case 1 (Fig. 2, lane G) and in Brodmann area 4 (frontal motor cortex) from case 2 (Fig. 2, lane M) while Brodmann area 4 from case 1 (Fig. 2, lane E) and Brodmann area 39 from case 2 (Fig. 2, lane N) were unaffected (Fig. 2). Several subcortical nuclei were also involved (see also Table 1). Cases 3–5 were more strongly affected, with a more intense doublet at 55 and 64 kDa (Fig. 2, lanes 1, R, S; Table 1). All of the regions studied contained the characteristic PiD Tau doublet. In all of the cases, Tau 69 was present in very low amounts. Twenty different regions corresponding to the different gyri of the frontal cortex were subsequently analyzed in cases 1 and 2. They all contained the doublet Tau 55, 64 (data not shown). The correlation between the presence of Pick bodies detected immunohistochemically and the PiD Tau doublet quantified biochemically was highly significant statistically: p < 0.0001; non-parametric Spearman correlation test.

The PiD Tau doublet differed from the typical PSP doublet (Fig. 2, lanes H, P), which comprises two bands at 64 and 69 kDa (32, 33), and from the 55, 64, 69 kDa Tau triplet that characterizes AD (29, 30) (Fig. 2, lanes A, Q). The pathological Tau proteins were quantified by densitometry and results compared to those obtained in AD (30) and PSP brain tissues (33). The amount of immunodetected Tau proteins in cases 1 and 2 was roughly 20% of the value obtained for AD and similar to that observed in PSP. It was 50% of that in case 3 and similar to those found in AD for cases 4 and 5. For cases 4 and 5, the number of Pick bodies was higher than in cases 1 and 2, as well as AD2 immunoreactivity (see Table 1).

Two-dimensional Analysis of Pathological Tau Proteins in PiD

Two-dimensional gel electrophoresis-western blot (2D blot) analysis of brain homogenates was performed using the isoelectric focusing protocol described by O’Farrell (42) and AD2 or Tau-1. First, a 2D blot analysis protocol was set up, using different brain areas from 10 AD patients. Results were highly reproducible. The AD case used in the present study showed a typical bidimensional electrophoresis profile. Carbamylated creatine phosphokinase was resolved as 34 spots in a linear range from pH 4.9 to 7.1 (Fig. 3A).

The 2D blot profile, which was the most representative in our study of 10 AD brains, is presented in Fig. 3. Pathological Tau proteins from the AD case, with an acidic isoelectric point ranging from 5.9 to 6.7, were clearly detectable with AD2 antibody. Four groups of proteins were immunostained (Fig. 3B). They corresponded to the characteristic Tau triplet with a MW of 55, 64 and 69 kDa (29, 30). The fourth group corresponded to spots at 74 kDa (small arrows, Fig. 3B), which were not detectable on monodimensional gels (Fig. 2, lanes A, Q). This 74 kDa group of proteins, which ended with well-focused spots in the acidic region at pH 6.0, is generally observed as a rule in AD brains with very large amounts of pathological Tau proteins and may represent...
Fig. 2. Western blot analysis of Tau profiles in different brain areas from 5 cases with Pick Disease (PiD). Comparison with homogenates of temporal cortex in an AD brain, putamen (put) and Brodmann area 4 of a PSP case. Pathological Tau proteins were detected with the monoclonal antibody AD2. Ten μl of homogenates of all brain areas studied were loaded in each well. The 55, 64, 69 kDa Tau triplet was detected in AD and the 64, 69 kDa doublet in PSP brain extracts. In all PiD brain tissues, a 55 and 64 kDa doublet is detected in the hippocampus, the frontal and temporal cortex. In case 1, the parietal cortex (area 7) was also affected, while case 2 displayed the PiD doublet in the motor cortex (area 4). Several subcortical nuclei showed the same abnormality (not shown). Cases 3 to 5 contained higher amounts of pathological Tau proteins, Tau 55, 64.

Minor components masked by smears on monodimensional SDS-PAGE (Fig. 2).

Using the same approach, two brain areas were studied in the 5 PiD brains: the frontal and temporal cortices. The parietal cortex of case 1 was also examined. The 2D blot profile was similar in all abnormal PiD brain areas studied (Fig. 3C). It differed significantly from those observed in AD brain samples (Fig. 3B). First, the 74 kDa group was not present in PiD. Second, the 69 kDa acidic spots, which were strongly detected in AD, were very faintly immunostained or not detected in PiD. In addition, the 64 kDa Tau pattern from PiD samples was focused as a large double spot (MW of 63 and 64 kDa) with a pI ranging from 6.85 to 6.4. The 55 kDa Tau protein of AD...
and PiD were similar, but the immunostaining in PiD was weaker. Three 54 kDa spots showing an isoelectric point ranging from pH 5.8 to pH 5.7 were specifically detected in PiD.

At last, the same blot (Fig. 3C) was also analyzed with Tau-1, a monoclonal antibody which detects Tau proteins that are not phosphorylated at Serines in the region 192-204 (40). Tau proteins with a less acidic isoelectric point and a lower MW were detected. The basic part of the 55 kDa was detected, with an acidic pi ending at 6.75 (Fig. 3D), but the 64 kDa major band and the 69 kDa were not labeled.

**DISCUSSION**

The Tau protein profile in 5 brains from PiD cases was determined with a highly specific and sensitive monoclonal antibody, AD2, which binds to a phosphorylation site located in the carboxy-terminal part of the Tau molecule (31, 37, 38). Post-mortem delays did not affect our biochemical study of pathological Tau proteins. After prolonged post-mortem delays (over 2 h), phosphatases completely dephosphorylate normal Tau at the AD2 and many other sites (30, 31, 43, 44), which renders them undetectable. Conversely, pathological Tau proteins bound to neuronal inclusions resist dephosphorylation by phosphatases and are still phosphorylated and immunodetected by AD2, even if post-mortem delays are longer than 96 h (31). Thus, AD2 immunolabeling in brain homogenates appears to represent a reliable and easy method to quantify pathological Tau proteins and neurofibrillary degeneration (33). This approach was used with success for the characterization of the Tau triplet in AD (29, 30) and Guamanian ALS/PDC (31) as well as for the Tau doublet 64 and 69 from PSP (32, 33). Kisezak-Reding et al observed a similar Tau profile in PSP and CBD (25).

Using non-dimensional SDS gels and western blots, Tau variant profiles were determined in several brain areas of five PiD brains. All affected areas showed the same Tau profile characterized by the 55 and 64 kDa doublet. Using SDS-PAGE, the PiD doublet has the same mobility as the two lowest proteins of the AD Tau triplet. The PiD doublet was principally observed in frontal and temporal neocortex and in the hippocampal formation. Biochemical results are in agreement with neuropathological findings, both showing the involvement of the association fronto-temporal cortices, the hippocampal formation, as well as subcortical nuclei (especially the striatum). However, morphological evaluation of the substantia nigra failed to reveal significant lesions in case 2, while mild loss of pigmented neurons with pigment incontinence without Lewy bodies were observed in case 1. The distribution of the PiD Tau doublet was heterogeneous since it was detected in the parietal areas of case 1, which displayed higher PB counts than case 2, and in the motor cortex of case 2. The amounts of pathological Tau proteins measured in affected brain areas from cases 1 and 2 were lower than in AD, but comparable to PSP. However, cases 4 and 5 contained much larger amounts, comparable to AD. In parallel to the immunodetection of the PiD doublet, we observed an increased amount of PB. It should be noted that NFT densities were particularly low in all of the Pick cases, demonstrating that the Tau 55, 64 doublet is specific to Pick's disease and Pick bodies. Similarly, we observed a highly significant correlation between the presence of PB and the amounts of Tau 55, 64 doublet. Altogether, our results show that each neurodegenerative disease analyzed displayed a specific profile of pathological Tau components: Tau 55, 64, 69 in AD, Tau 64, 69 in PSP or CBD, and Tau 55, 64 in PiD.

Closer comparison of those components by 2D blots that are highly resolutive revealed similarities and differences between AD and PiD. In PiD, after AD2 immunolabeling, the 74 kDa group was absent, the 69 kDa group was faint, and the 64 kDa was focused as a large spot in a less acidic region. On the other hand, the 55 kDa from AD and PiD was similar in terms of isoelectric point but its immunodetection was weaker in PiD. One additional group consisting of 3 acidic spots was detected specifically at 54 kDa in PiD frontal cortex. These two components (54 and 55 kDa) may correspond to hyperphosphorylation of the two shortest Tau isoforms (45). The 64 kDa component is the most characteristic Tau component from PiD. A detailed examination shows that this spot is composed of two close bands at MW 63 and 64 kDa and corresponds to pathological Tau isoforms because its presence is closely related to the presence of PB. The AD2 staining of the 64 kDa double band demonstrates that it is phosphorylated in the carboxy terminal part of Tau. Similarly, the absence of labeling with Tau-1 is in favor of a phosphorylation at one or several Serine motifs at 192-204 region of Tau. The 64 kDa double band strongly immunodetected in PiD likely corresponds to the two intermediate isoforms described by Goedert et al with exon 2 either alone (isoform 381) or associated with exon 10 (isoform 412) (45). These suggestions are based on the fact that we observe the same molecular weight species as in AD, and that differences are only seen at the level of isoelectric points and Tau concentrations.

In conclusion, pathological Tau proteins in AD, PSP/CBD and PiD display different and characteristic patterns. The pathophysiological mechanisms responsible for those molecular differences are not fully determined. Among possible explanations, selectively vulnerable neuronal subpopulations involved in each disease may express specific Tau isoform stoichiometric ratios (12, 13, 25). The neuronal subsets vulnerable in PiD could normally express very little of the heaviest isoforms corresponding to the 69 kDa band seen in AD (29–32, 45).
A Carbamylytes

B Alzheimer, AD2

C Pick, AD2

D Pick, Tau1
At the molecular levels, specific dysfunctions of kinase and/or phosphatase enzymatic cascades which regulate Tau phosphorylation may also be differently involved in PiD and AD (43, 44, 46).

Our results demonstrate that PB constitute a molecular type of neurofilamentous neuronal inclusion characterized at the molecular level by a specific Tau profile and distinct from that seen in AD and PSP. Thus, pathological Tau proteins may be used for the molecular mapping of neurofibrillary degeneration as already shown in AD (30), normal aging (47), Parkinsonism with dementia (48), PSP (33), and the Guamanian ALS/PDC (31). These methods may also be applied to brain biopsies, and have already proven to be helpful in several cases (49); alternatively, they may be used to monitor expression of Tau protein isoforms in cell culture systems (50), animal models of aging (51), or transgenic mice using promoters of cytoskeletal proteins.

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< Fig. 3. Two-dimensional analysis of pathological Tau proteins in brain homogenates. A five µl of carbacholylates were added to the SDS protein sample to determine the pH gradient and to check its linearity. The gel stained with Coomasie Blue revealed the good linearity of the pH gradient and the good resolution of carbachylated creatine phosphokinase which is focused as 34 spots ranging from pH 7.1 to 4.9, as expected from the manufacturer's instructions. B. Analysis of temporal cortex in an AD brain (Area 22). Fifteen µl of brain homogenate were loaded. Pathological Tau proteins were specifically immunostained by monoclonal antibody AD2. Four groups of Tau proteins were detected, all marked by arrows. The most characteristic bands are the spots at 74 kDa (small arrows), the extended band of proteins at 69 kDa, the well-focused spots at the acidic end of the 64 kDa group and the large band at 55 kDa. C. Analysis of a frontal cortex sample in a PiD brain. Twenty µl were loaded and the 2D blot was exposed a longer time in order to reveal minor bands. Four groups of Tau proteins were detected. The 69 kDa group was faintly detected. The 64 kDa band was mainly focused in the less acidic region. It is composed of two bands (63 and 64 kDa, marked by small arrows). The 55 kDa was similar to the one observed in AD. Three spots at 54 kDa were detected in the acidic region in PiD samples. D. Immunoblotting of Tau proteins from the same PiD sample (C) with Tau-1, after removal of AD2 staining by stripping. Three basic bands were stained. The 55 kDa corresponds to the more basic portion of the 55 kDa band detected by AD2. Note that the 64 kDa band well labeled with AD2 is not detected with Tau-1. In order to facilitate a comparison between the 2D blots (B, C, D) which are at the same scale, dotted lines were drawn on each major bands.>
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