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Symposium: Tauopathies: Update and Perspectives

Biochemistry and molecular biology of tauopathies

Masato Hasegawa

Department of Molecular Neurobiology, Tokyo Institute of Psychiatry, Tokyo Metropolitan Organization for Medical Research, Tokyo, Japan

Filamentous tau deposits in neurons or glial cells are the hallmark lesions of neurodegenerative tauopathies, such as Alzheimer's disease, Pick's disease, corticobasal degeneration and progressive supranuclear palsy. Biochemical analyses of Sarkosyl-insoluble tau from brains with tauopathies have revealed that tau deposits in different diseases consisted of different tau isoforms (i.e., all six tau isoforms occur in Alzheimer's disease, four repeat tau isoforms occur in corticobasal degeneration or progressive supranuclear palsy, and three repeat tau isoforms occur in Pick's disease). The discovery of mutations in the tau gene in FTDP-17 has established that abnormalities in tau function or expression are sufficient to cause filamentous aggregation of hyperphosphorylated tau and neurodegeneration similar to that seen in sporadic tauopathies. Because the number of tau inclusions and their regional distribution correlate with clinical symptoms, inhibition of tau aggregation or filament formation in neurons or glial cells may prevent neurodegeneration. We have investigated the effects of 42 compounds belonging to nine different chemical classes on tau filament formation, and found that several phenothiazine and polyphenol compounds, and one porphyrin compound inhibit tau filament formation.

Key words: Alzheimer's disease, corticobasal degeneration, FTDP-17, Pick's disease, progressive supranuclear palsy, tau.

INTRODUCTION

Accumulation of hyperphosphorylated tau proteins in neurons or glial cells is the hallmark lesion of many neurodegenerative diseases, including Alzheimer's disease (AD), Pick's disease (PiD), corticobasal degeneration (CBD) and progressive supranuclear palsy (PSP), collectively referred to as tauopathies. The number of tau inclusions and their regional distribution correlate with clinical symptoms in some neurodegenerative diseases. The biological significance of tau inclusions was demonstrated in 1998 by the discovery of mutations in the tau gene in frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17).

Analysis of the effects of tau mutations and tau deposits in FTDP-17 patients by us and other groups, has established that abnormalities in tau function or expression are important in causing filamentous aggregation of hyperphosphorylated tau and neurodegeneration.[1] The molecular mechanisms underlying tau accumulations in sporadic tauopathies remain to be clarified; however, inhibition of tau aggregation or filament formation in neurons or glial cells may prevent neurodegeneration.

We investigated the effects of 42 compounds, belonging to nine different chemical classes, on tau filament formation and found that several phenothiazine and polyphenol compounds and one porphyrin compound inhibit both tau filament formation and Aβ aggregation.[2]

In the present paper, I review recent progress in understanding both the biochemistry and molecular biology of tauopathies and therapeutic strategies that target these diseases.

EXPRESSION OF TAU PROTEIN ISOFORMS

Tau is one of the microtubule-associated proteins that promotes the polymerization of tubulin into microtubules and stabilizes microtubules. The human tau gene is located on the long arm of chromosome 17, and encodes the expression of six tau isoforms in the central nervous system. These six isoforms differ by the presence or absence of a 31 amino acid insert in the C-terminal region, encoded by exon 10, in conjunction with the presence or absence of a 29 or 58 amino acids inserts in the N-terminal domain, coded by exon 2 or exons 2 and 3, respectively (Fig. 1A).[3,4] Deletion or insertion of the cassette exon 10 produces tau

Correspondence: Masato Hasegawa, PhD, Department of Molecular Neurobiology, Tokyo Institute of Psychiatry, 2-1-8 Kamikitazawa, Setagaya-ku, Tokyo 156-8585, Japan. Email: masato@prit.go.jp

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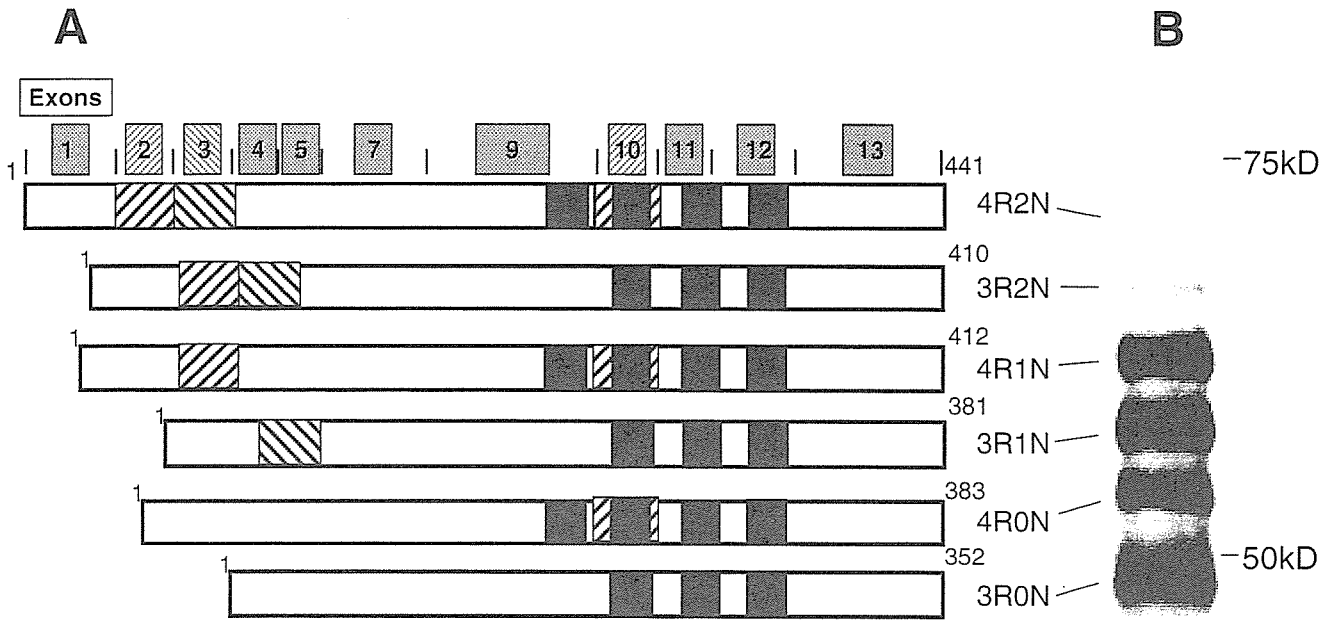

Fig. 1 Six tau isoforms in human adult brain. (A) Schematic diagram of the six tau isoforms expressed in adult human brain. Alternatively spliced exons (cassette exons) are shown in diagonal lines and microtubule binding repeats are shown in black. (B) The six tau isoforms are visible on an immunoblot of dephosphorylated soluble tau from a control case labeled with HT7.

isoforms with three repeats (3R tau) or four repeats (4R tau), respectively. The repeat sequences in the C-terminal region of tau bind to tubulin and promote microtubule assembly. The 4R isoform promotes microtubule assembly at a rate about 2.5-fold higher than the 3R tau isoform.

The expression of tau isoforms is developmentally regulated. In fetal or juvenile stages in mammals only the shortest tau isoform with no insert (3R tau) is expressed, whereas during development the cassette exons are inserted and multiple tau isoforms are expressed in the adult brain. In the rodent brain, the 3R tau isoform is completely replaced by the 4R tau isoform in the adult, whereas in the human brain the 3R tau isoform is expressed throughout life. Significantly, this is determined by the DNA sequence in the exon 10 splice donor site, where several tau mutations have been identified in FTDP-17.[5] The expression of the 3R tau isoform in the adult human brain may be related to neuronal plasticity and higher brain function.

The human six tau isoform is detected at around 48–68 kDa by immunoblotting of dephosphorylated soluble fractions from adult human brains with a phosphorylation-independent anti-tau antibody, such as HT7 (Fig. 1B). Tau isoforms with an N-terminal 29 amino acid insert (1N) appear as the strongest bands, followed by those with no N-terminal insert (0N), while tau isoforms with N-terminal 58 amino acids inserts (2N) are weakly detected (Fig. 1B), suggesting that expression levels of the 2N isoforms are low. The expression levels of the 3R tau isoform is 1–1.5-fold higher than the expression level of the 4R tau isoform (Fig. 1B).[6]

Expression of the 3R tau and 4R tau isoforms is regulated in a manner that is cell-type specific. *In situ* hybridization has shown that transcripts of the 3R tau and 4R tau isoforms are expressed in pyramidal cells of the subiculum and hippocampus, whereas only the transcript of the 3R tau isoforms is found in granule cells of the dentate gyrus.[7] As described below, 3R tau isoform deposits in PiD occur in these granule cells as Pick bodies. The 4R tau isoform may be the major isoform expressed in glial cells, because in most cases studied tau deposits in glial cells were the 4R tau isoform, although there is no *in situ* hybridization data to support this.

TAU PROTEIN DEPOSITS IN BRAINS WITH TAUOPATHIES

To biochemically investigate tau protein deposits from the brains of individuals with tauopathies, Sarkosyl-insoluble tau was prepared from frozen brain tissue of AD, CBD, PSP and PiD brains with characteristic pathologies (Fig. 2). The frontal cortex was homogenized in 10-fold (v/w) extraction buffer (10 mM Tris-HCl (pH 7.5), 1 mM EGTA, 0.8 M NaCl, 10% sucrose) and centrifuged at 23 000 × *g* for 20 min at 4°C. The pellet was sequentially extracted by homogenization in 1% Triton (Sigma-Aldrich, St Louis, USA), 1% Sarkosyl and 7 M guanidine-HCl, followed by ultracentrifugation. The Sarkosyl-insoluble, guanidine-

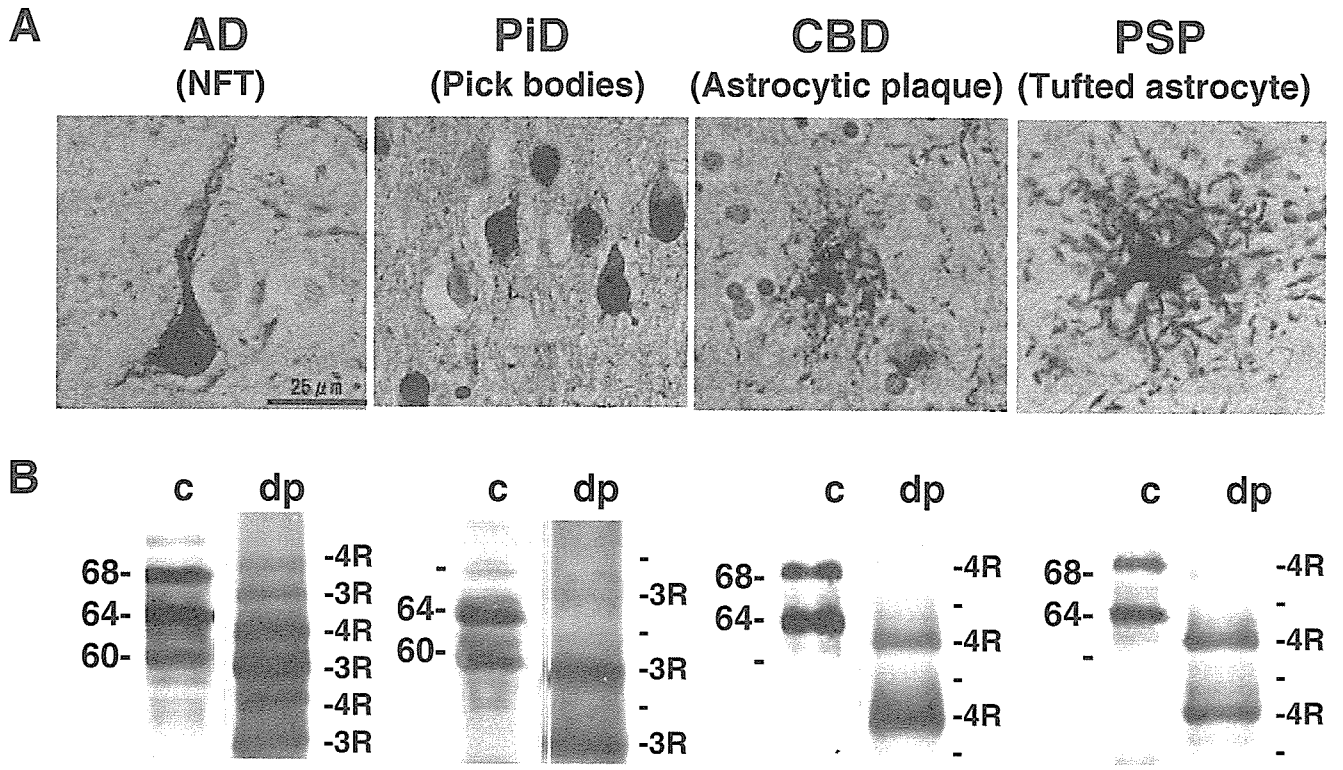

Fig. 2 (A) Characteristic tau pathologies in Alzheimer's disease (AD), Pick's disease (PiD), progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD) as visualized with AT8: a neurofibrillary tangle (NFT) can be seen in AD, Pick bodies in PiD, astrocytic plaque in CBD and a tufted astrocyte can be seen in PSP. (B) The Sarkosyl-insoluble tau band patterns before and after dephosphorylation: immunoblot analysis of Sarkosyl-insoluble tau before (c) and after (dp) alkaline phosphatase treatment with anti-tau antibody HT7.

HCl-soluble fraction was dialyzed against 30 mM Tris-HCl (pH 8.8) and treated with alkaline phosphatase at 67°C.[6] Samples were separated by SDS-PAGE and immunoblotted with an anti-tau antibody HT7. The Sarkosyl-insoluble tau from AD brains consisted of a triplet of bands with apparent molecular masses of 60 kDa, 64 kDa and 68 kDa, which resolved into six bands after dephosphorylation (Fig. 2B). The Sarkosyl-insoluble tau from PSP and CBD brains appeared as two major bands of 64 kDa and 68 kDa, whereas that from PiD brains was visible as two major bands of 60 kDa and 64 kDa. After dephosphorylation, the Sarkosyl-insoluble tau bands from PSP or CBD brains resolved into two major bands plus one band corresponding to the 4R tau isoform. After dephosphorylation, the tau bands from PiD brains resolved into two major bands and one minor band that correspond to the 3R tau isoform. These results are consistent with previous observations (Fig. 2B). We examined at least eight cases of AD, four cases of CBD, seven cases of PSP and six cases of PiD, and obtained similar results.[6]

Recently, monoclonal antibodies, RD3 and RD4, which specifically recognize the 3R tau isoform and the 4R tau isoform, respectively, were generated by de Silva *et al.* and some applications of these antibodies have been reported.[8,9] These antibodies are now commercially available from Upstate cell signaling solutions (VA, USA). We tested these antibodies in immunoblot analysis of Sarkosyl-insoluble tau from AD, PiD, CBD and PSP brains (Fig. 3). The RD3 antibody labeled dephosphorylated Sarkosyl-insoluble tau bands, which corresponded to the 3R tau isoform, from AD and PiD brains, but did not label any bands from CBD and PSP brains. In contrast, the RD4 antibody labeled Sarkosyl-insoluble tau bands from AD and CBD brains but no immunoreactivity was detected on the immunoblot of PiD and PSP brains (Fig. 3). The RD4 failed to detect the insoluble tau bands from this PSP case, probably due to the low amount of tau, which was barely detectable with HT7 antibody. The specificity of these antibodies was confirmed on immunoblots of the recombinant human 6 tau isoform (Fig. 3). These results support previous observations and suggest that these antibodies may be very useful for detecting the presence of 3R tau or 4R tau isoforms. It is impossible to isolate and characterize the tau deposit from one neuron or one glial cell using biochemical extraction methods. However, these antibodies may be powerful tools for localizing the 3R tau and 4R tau iso-

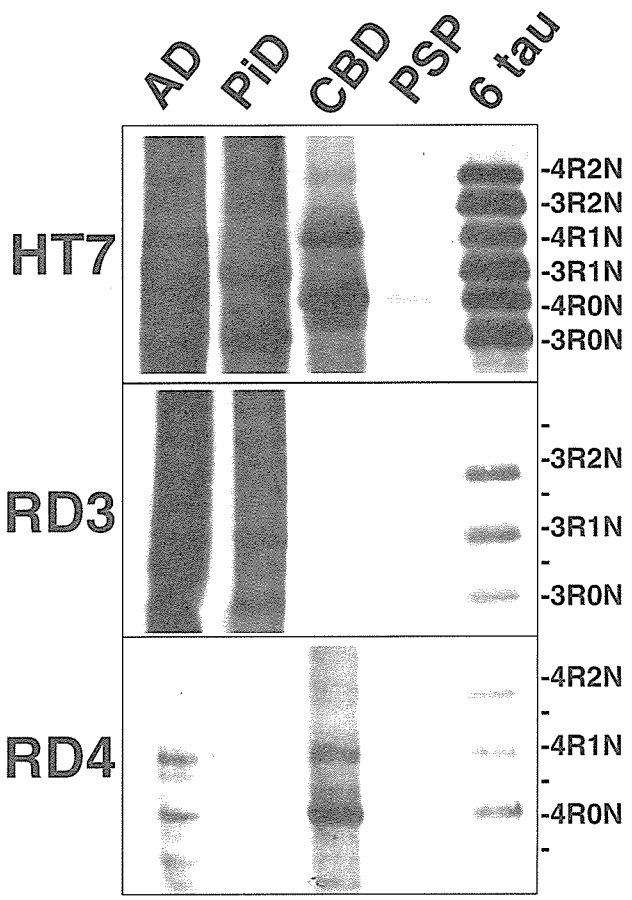

Fig. 3 Immunoblot analysis of tau isoforms with RD3 and RD4 antibodies. Dephosphorylated Sarkosyl-insoluble tau from Alzheimer's disease (AD), Pick's disease (PiD), corticobasal degeneration (CBD) and progressive supranuclear palsy (PSP) brains, and six recombinant human tau isoforms were immunoblotted with HT7, RD3 and RD4 antibodies. RD3 and RD4 specifically recognized the 3R tau isoform and the 4R tau isoforms, respectively.

forms to specific cell types, although further immunohistochemical studies with these antibodies are needed.

Corticobasal degeneration and PSP brains have distinctive pathological features; however, no biochemical differences in tau deposits have been identified. Arai *et al.* examined the brains of patients with PSP and CBD and identified a difference in the tau band patterns between CBD and PSP on immunoblots for Sarkosyl-insoluble tau.[10] In PSP brains a 33 kDa tau band predominated, whereas two closely related bands of about 37 kDa predominated in CBD brains. Immunoblots of one atypical case with pathological features of both CBD and PSP showed both bands. Furthermore, protein sequencing and immunochemical analyses showed that the 33 kDa band and the 37 kDa doublet consisted of the carboxyl half of the tau protein with different amino termini.[10] Although the mechanism underlying tau processing in PSP and CBD remains to be clarified, this report suggests that the biochemical divergence may be related to the neuropathological features of these diseases and that distinctive inclusions can be distinguished by a proper biochemical detection method. Related to this, we have succeeded in distinguishing the tau deposits in CBD, PSP and FTDP-17 with an intronic mutation by detecting protease-resistant tau bands (data not shown).

EXPRESSION OF TAU ISOFORMS IN SPORADIC TAUOPATHIES

The discovery of exonic and intronic mutations in the tau gene in FTDP-17 has shown that abnormalities in tau mRNA splicing, or dysfunction of the tau protein, are sufficient to cause neurodegeneration. To date, more than 30 different mutations have been reported in patients with FTDP-17. Exonic mutations occur in or near to the microtubule-binding domain, except for two missense mutations. Most of the exonic mutations affect the ability of the tau protein to promote microtubule assembly,[11] and some affect the propensity of aggregation,[12] whereas the intronic mutations, and silent mutations and some missense mutations in exon 10, affect mRNA splicing of exon 10.[13,14]

By analogy with FTDP-17, alterations in the relative ratio of transcripts of the 3R tau and 4R tau isoforms have been suggested to occur in PSP or CBD,[15,16] where the 4R tau isoform is deposited in affected regions. However, it is unclear whether there is any actual change in the mRNA splicing and overproduction of the 4R tau isoform in the brains of individuals with PSP or CBD. It is also unclear whether there is any alteration in tau mRNA expression or the expression of tau proteins in the brains of individuals with PiD where the 3R tau isoform accumulates in neuronal cells in affected regions of the brain.

We have analyzed the expression of tau transcripts and tau protein isoforms in brains with sporadic tauopathies, including PSP, CBD, PiD and AD, by RT-PCR (Fig. 4).[6] In control cases, almost equal levels of tau mRNA transcripts without E10 (corresponding to 3R tau mRNA) and tau mRNA transcripts with E10 (corresponding to 4R tau mRNA), or slightly higher levels of 3R tau mRNA than 4R tau mRNA, were detected (Fig. 4A). In cases with sporadic AD, variable patterns were observed. In cases with PSP and CBD, similar levels of 3R tau mRNA and 4R tau mRNA, or increased ratios of 4R tau mRNA, were detected. Increased levels of 4R tau mRNA compared to 3R tau mRNA were also detected in four out of six cases with PiD (Fig. 4A).[6] Significantly, low expression or no expression of neurofilament medium size (NF-M) mRNA was observed in sporadic cases of tauopathy where 3R tau mRNA levels decreased (Fig. 4A). When the levels of each transcript were plotted, there was a strong correlation

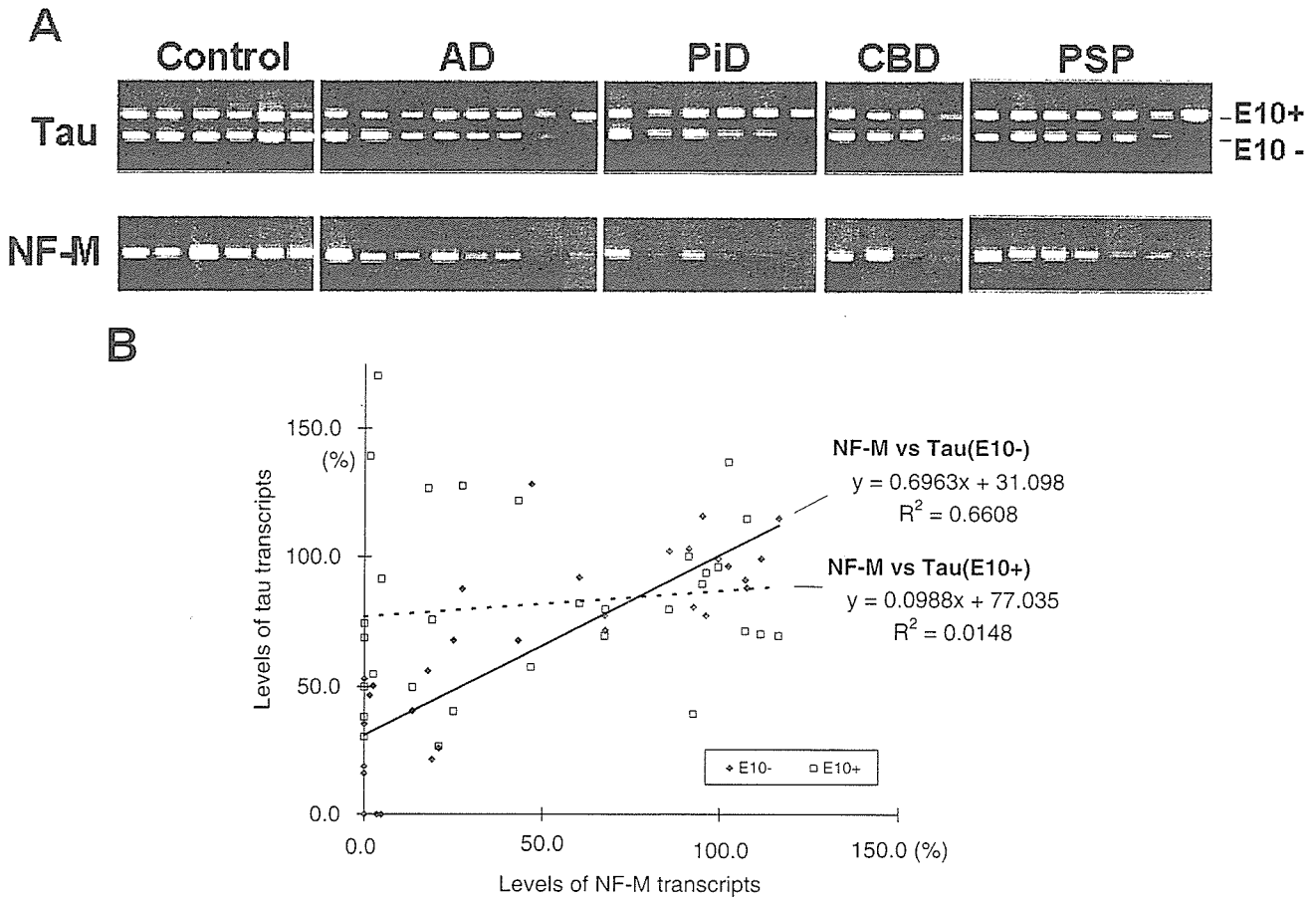

Fig. 4 (A) RT-PCR analysis of tau transcripts and neurofilament medium size (NF-M) transcripts in control brains and tauopathy brains. Correlation of tau transcript levels with NF-M transcript levels. A strong correlation between the levels of the 3R tau (E10-) transcript and NF-M transcript was found; however, no correlation was found between the levels of the 4R tau (E10+) transcript and NF-M transcript. (B) Ethidium bromide-stained agarose gel of PCR products of tau and NF-M from control, Alzheimer's disease (AD), Pick's disease (PiD), corticobasal degeneration (CBD) and progressive supranuclear palsy (PSP) brains. A typical experiment is shown; similar results were obtained in three separate experiments.

between the levels of 3R tau transcripts and NF-M transcripts (Fig. 4B), whereas there was no correlation between the levels of 4R tau transcripts and NF-M transcripts (Fig. 4B).[6] These results suggest that a decreased level of 3R tau mRNA (or an increase in the relative ratio of 4R tau mRNA/3R tau mRNA) is associated with a decreased level of NF-M mRNA, indicating that the increase in the relative ratios of 4R tau/3R tau at the mRNA levels in sporadic cases of PSP or CBD, as well as in sporadic cases of PiD or AD, may largely be due to a decrease in 3R tau transcripts associated with neuronal loss or neurodegeneration.

It is not known why distinct tau isoforms with characteristic tau inclusions accumulate in different regions of the brain in these sporadic tauopathies. One possible explanation is that tau deposition in the degenerating cell may simply reflect the expression patterns of tau isoforms in the cell, which are different in each subset of neurons or glial cells according to cell type or anatomical area.

INHIBITION OF TAU FILAMENT FORMATION

The conversion of soluble peptides and proteins into insoluble filaments is believed to be the central event in the etiology of the most common neurodegenerative diseases.[1] Therefore, current therapeutic strategies aim to inhibit protein aggregation and promote clearance of the insoluble amyloid proteins Compared with the number of studies on extracellular protein deposits, such as Aβ or prion protein, there are only a few reports on therapeutic strategies aimed at inhibiting tau filament formation.

We investigated the effects of 42 compounds, belonging to nine different chemical classes, on heparin-induced tau filament formation. Filament formation was assessed using electron microscopy, thioflavin S (ThS) fluorescence and the formation of Sarkosyl-insoluble tau protein. Several phenothiazines (methylene blue, azure A, azure B and quinacrine mustard), polyphenols (myricetin, epicatechin

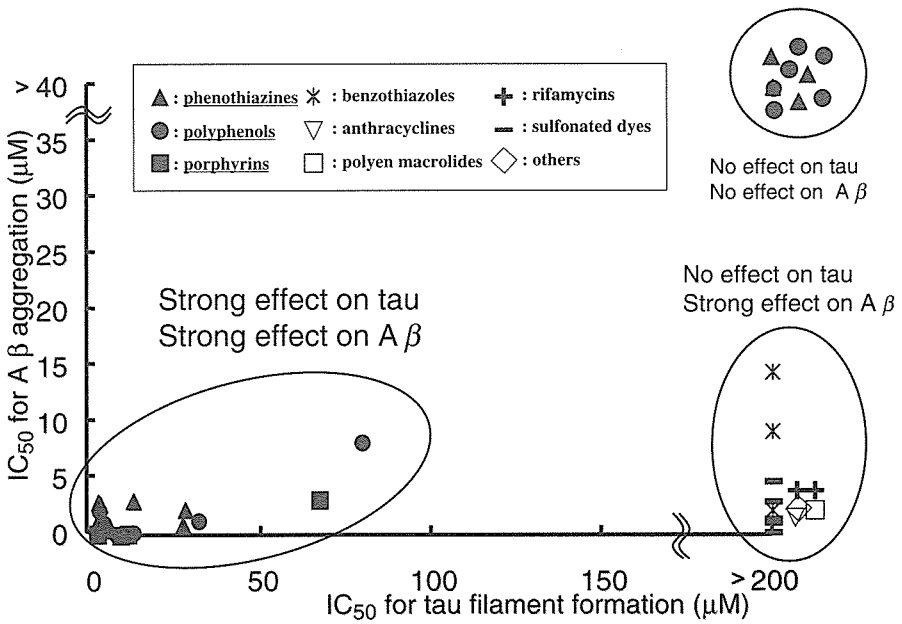

Fig. 5 IC_{50} values of 42 compounds from nine different chemical classes for inhibition of Aβ aggregation and tau filament formation. A strong inhibitory effect on both tau and Aβ fibril formation was observed in several compounds belonging to the phenothiazine, polyphenol and porphyrin classes.

3-gallate, gossypetin and 2,3,4,2′,4′-pentahydroxyphenone) and the porphyrin ferric-dehydroporphyrin IX inhibited both tau filament formation and Aβ aggregation, with IC_{50} values in the low micromolar range (Fig. 5).[2] In contrast to the significant number of compounds that inhibited tau assembly, only the porphyrin phthalocyanine was able to disassemble tau filaments. Analysis of the Sarkosyl-soluble fraction of tau assemblies by SDS-PAGE revealed large amounts of HMW tau in the presence of inhibitory compounds.[2]

These findings, together with other results, suggest that oligomeric tau may be an intermediate in the pathway from monomeric to filamentous tau and that the compounds may be inhibitory towards tau assembly by virtue of their ability to bind to and stabilize tau dimers or oligomers. Phenothiazine compounds are known to cross the blood–brain barrier and are relatively non-toxic. The identification of phenothiazine, polyphenol and porphyrin compounds as inhibitors of tau filament formation forms a starting point for the development of mechanism-based therapies for the tauopathies.

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CASE REPORT

Corticobasal degeneration with focal, massive tau accumulation in the subcortical white matter astrocytes

Kenji Sakai · Yue-Shan Piao · Koki Kikugawa · Shinji Ohara · Masato Hasegawa · Hiroki Takano · Masayuki Fukase · Masatoyo Nishizawa · Akiyoshi Kakita · Hitoshi Takahashi

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Abstract We report two sporadic cases of tauopathy with unusual neuropathological features. The ages of the patients at death were 86 and 74 years, and the disease durations were 4 and 3 years, respectively. The former patient showed progressive dementia and amyotrophy (autopsy revealed that severe cervical spondylosis was responsible for the amyotrophy), and the latter showed progressive parkinsonism and dementia. The essential brain pathologies were similar to each other; although ballooned neurons and astrocytic tau lesions (astrocytic plaques) were present in the affected cerebral cortex, the most striking finding was focal, much heavier accumulation of tau in the subcortical white matter. Moreover, double-labeling immunostaining, as well as Gallyas–Braak electron and AT8 immunoelectron microscopic studies strongly suggested that in the affected subcortical white matter, the accumulation of tau occurred mainly in the astrocytic processes. In the latter patient, for whom frozen brain tissue was available, immunoblotting of insoluble tau revealed a pattern compatible with that obtained from brain affected by typical corticobasal degeneration (CBD), and gene analysis of tau revealed no mutations, with a H1 haplotype. Finally, in both cases, the pathological diagnosis of CBD was considered to be appropriate. However, the tau pathology affecting the subcortical white matter astrocytes was very unusual for the disease.

Keywords Tauopathy · Corticobasal degeneration · Dementia · Subcortical white matter · Astrocyte

K. Sakai · H. Takahashi (✉)
Department of Pathology, Brain Research Institute,
University of Niigata, 1-757 Asahimachi,
Niigata 951-8585, Japan
e-mail: hitoshi@bri.niigata-u.ac.jp

Y.-S. Piao
Department of Neuropathology, Xuanwu Hospital,
Capital University of Medical Sciences,
45 Chang Chun Street, Beijing 100053,
People's Republic of China

K. Kikugawa · M. Fukase
Departments of Neurology and Pathology,
Shonai Hospital, 4-20 Izumimachi,
Tsuruoka 997-8515, Japan

S. Ohara
Department of Neurology,
National Chushin-Matsumoto Hospital,
811 Kotobuki, Matsumoto 399-0021, Japan

M. Hasegawa
Department of Molecular Neurobiology,
Tokyo Institute of Psychiatry, Tokyo Metropolitan
Organization for Medical Research, 2-1-8 Kamikitazawa,
Setagaya-ku, Tokyo 156-8585, Japan

H. Takano · M. Nishizawa
Department of Neurology, Brain Research Institute,
University of Niigata, Niigata 951-8585, Japan

A. Kakita
Department of Pathological Neuroscience,
Resource Branch for Brain Disease Research CBBR,
Brain Research Institute, University of Niigata,
Niigata 951-8585, Japan

Present Address:
K. Sakai
Department of Neurology and Neurobiology of Aging,
Kanazawa University Graduate School of Medical Science,
13-1 Takara-machi, Kanazawa 920-8640, Japan

Introduction

Corticobasal degeneration (CBD), which is a sporadic neurodegenerative disorder of mid to late adult life, is often characterized clinically by progressive levodopa-nonresponsive parkinsonism with focal cortical signs, such as apraxia and aphasia, and progressive dementia of the frontotemporal type [18]. The major neuropathological abnormalities include asymmetrical frontoparietal atrophy, the presence of ballooned neurons within the affected cerebral cortex, and degeneration of the subcortical nuclei (especially the substantia nigra and globus pallidus). The most distinctive cytopathological feature of CBD is the presence of widespread tau pathology affecting both neuronal and glial (oligodendroglial and astrocytic) cells; the disease is now well known to be one of the sporadic tauopathies [7, 17]. With regard to the astrocytic tau lesions, there are two well recognized morphologies, namely, tufted astrocytes (TAs) and astrocytic plaques (APs); importantly, it has been considered that TAs occur almost exclusively in progressive supranuclear palsy (PSP), another sporadic tauopathy [11], whereas APs, which consist of an annulus of tau-positive structures surrounding a clear central core [10], are specific for CBD [7, 8, 16, 24]. Here we present two sporadic cases of tauopathy, in which focal, much heavier accumulation of tau in the subcortical white matter astrocytes was a characteristic feature.

Case reports

Neither of the affected patients had a family history of dementia or other neurological disease. The clinicopathology of one of the two cases (case 2) has been reported previously [19]. In the present study, we reexamined this case using additional new methods.

Case 1

The patient was an 86-year-old Japanese man. At the age of 81 years, he developed slowly progressive gait disturbance. He was found to have a cervical disc hernia and underwent a laminectomy (C3–C7). However, weakness of the upper extremities appeared and gait disturbance worsened. Five months later, he was admitted to our hospital for neurological evaluation. On examination, the patient was apparently demented: his score on the revised version of the Hasegawa dementia scale (HDS-R; a mental examination method commonly used in Japan that utilizes a scale of 0–30, where < 21 represents dementia) was 13. He showed disorientation as to date, and also memory and naming disturbances. Neurogenic muscular atrophy, hyperreflexia, positive plantar reflex, and gait disturbance were also evident. He exhibited no apraxia or aphasia. At the age of 83 years, brain magnetic resonance imaging (MRI) revealed diffuse symmetric cerebral cortical atrophy (Fig. 1). Cervical and lumbar MRI showed severe cervical spondylosis and lumbar spine canal stenosis, respectively. Thereafter, his dementia and muscular atrophy progressed gradually. Although careful consideration was given to the MRI findings, a tentative diagnosis of amyotrophic lateral sclerosis (ALS) with dementia was made. At the age of 84 years, he became bed-ridden. In the final year of his illness, he had repeated episodes of bronchopneumonia, and died at the age of 86 years, about 4 years after onset of the disease. A general autopsy was performed 2 h after death, at which time the brain weighed 1,040 g.

Case 2

In brief, the patient was a 74-year-old Japanese man. At the age of 71 years, he started to suffer from backward falls. Thereafter, bradykinesia and urinary incontinence gradually became apparent. On examination at the age of 74, the patient had a mask-like face and his speech was slow and monotonous. He was apparently demented: on the Mini-Mental State Examination, he scored 14 out of 30. He showed disorientation as to date, and day of the week, and short-term memory disturbance. He also exhibited aphasic agraphia and constructional apraxia. Truncal rigidity and increased deep tendon reflexes were evident (Babinski signs were negative). He was unable to get up from bed without assistance. No alien limb phenomenon or limb dystonia was observed. MRI revealed diffuse symmetric cerebral cortical atrophy. The cerebellum and brainstem were also atrophic. A tentative diagnosis of striato-nigral degeneration-type multiple system atrophy (MSA) was made. He died of extensive ischemic colitis, about 3 years after onset of the disease. A general autopsy was performed 1 h after death, at which time the brain weighed 1,040 g.

Materials and methods

Neuropathological examination

In the present study, formalin-fixed and paraffin-embedded tissue blocks of various brain and spinal cord regions were available in both cases. Histological

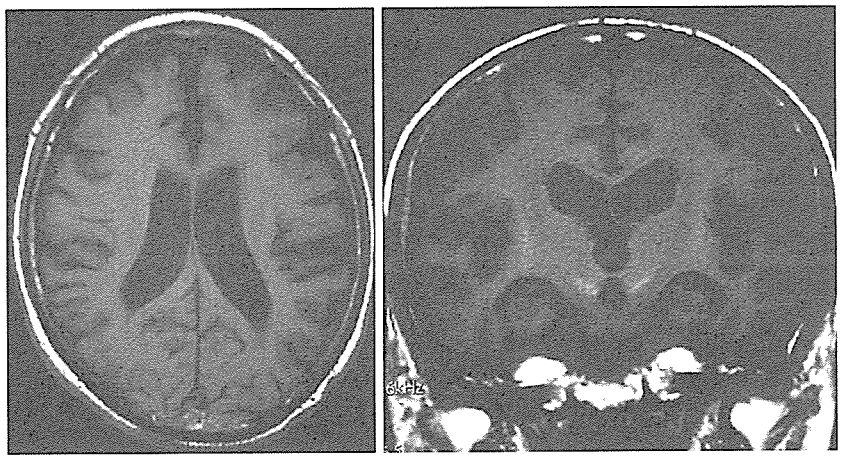

Fig. 1 T1-weighted MRI images obtained from case 1, showing symmetric, mild atrophy of the frontal and temporal lobes, with dilatation of the lateral and third ventricles

examinations were performed on 4-μm-thick sections using several stains: hematoxylin–eosin, Klüver-Barrera, Holzer and Gallyas–Braak. Selected sections were also immunostained using the avidin–biotin-peroxidase complex (ABC) method (Vector, Burlingame, CA, USA) with diaminobenzidine as the chromogen. The primary antibodies used were rabbit polyclonal antibodies against glial fibrillary acidic protein (GFAP; Dako, Glostrup, Denmark; 1:2,000) and ubiquitin (Dako; 1:800), and mouse monoclonal antibodies against β-amyloid (Dako; 1:100), phosphorylation-dependent tau (AT8; Innogenetics, Ghent, Belgium; 1:200) and phosphorylated α-synuclein (#64; Wako, Osaka, Japan; 1:10,000).

Furthermore, 7-μm-thick sections obtained from various brain regions in both cases were immunostained with specific mouse monoclonal antibodies against 3-repeat tau (RD3; Upstate, Charlottesville, VA, USA; 1:3,000) and 4-repeat tau (RD4; Upstate; 1:100) as described previously [6]. In addition, a double-labeling immunostaining study was performed on selected sections, with mouse monoclonal AT8 (1:300) and rabbit polyclonal GFAP (1:3000). Immunolabeling was detected using the ABC method and the ABC alkaline phosphatase method (ABC-AP Kit, Vector) and visualized with DAB and fast blue, respectively.

Argyrophilic lesions observed in the subcortical white matter (described below) were examined using the Gallyas–Braak electron microscopic method [3, 12]. Formalin-fixed small tissue blocks from the frontal subcortical white matter in case 2 were sectioned at 50 μm thickness using a vibratome. The sections were stained by the Gallyas–Braak method using the free floating technique, then postfixed with 3% glutaraldehyde and 1% paraformaldehyde, followed by staining with 1% osmium tetroxide, and embedded in Epon 812. Ultrathin sections were cut, and then stained with uranyl acetate and lead citrate. For immunoelectron microscopy of tau lesions observed in the subcortical white matter (described below), formalin-fixed small tissue blocks from the frontal subcortical white matter in case 1 were embedded in LR-White resin (London Resin, Reading, UK). Ultrathin sections were cut, placed on Collodion-coated nickel grids, incubated in AT8 (1:100), and reacted with a 15-nm gold colloidal particle-conjugated anti-mouse IgG (British BioCell, Cardiff, UK; 1:30). The sections were then stained with uranyl acetate and lead citrate. These ultrathin sections prepared from cases 2 and 1 were examined with a Hitachi H-7100 electron microscope at 75 kV.

Biochemical and gene analyses of tau in case 2

Sarkosyl-insoluble tau was prepared from the frozen frontal and temporal lobes, and midbrain, as well as from the frontal lobe of a typical CBD case and a typical PSP case, as described previously [2]. Tau protein was run on a 10% sodium dodecyl sulfate-polyacrylamide gel, transferred to a polyvinylidene difluoride membrane (Durapore membrane; Millipore, Billerica, MA, USA), and probed with a phosphorylation-independent anti-tau antibody, T46 (Zymed, South San Francisco, CA, USA; 1:2,000), and a phosphorylation-dependent anti-tau antibody, PS396 (Calbiochem, Darmstadt, Germany; 1:2,000).

Genomic DNAs were also prepared from the frozen fontal lobe sample using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). All coding exons expressed in the human central nervous system (exons 1, 2, 3, 4, 5, 7, 9, 10, 11, 12, and 13) of the tau gene were amplified by PCR with the flanking intronic sequences [21]. The nucleotide sequences were determined using an ABI3100 DNA sequencer (Applied Biosystems,

Foster City, CA, USA) and a DYEnamic ET terminator sequencing kit (Amersham Biosciences, Piscataway, NJ, USA).

Results

Neuropathological findings

In case 1, the brain showed symmetric frontal lobar atrophy (Fig. 2a). On sections, mild atrophy of the globus pallidus (Fig. 2b) and marked depigmentation of the substantia nigra were evident (Fig. 2c). In case 2, the brain showed diffuse brain atrophy, being more conspicuous in the cerebellum and brainstem. On sections, atrophy and brownish discoloration of the globus pallidus (especially the medial segment), subthalamic nucleus, and cerebellar dentate nucleus, and marked depigmentation of the substantia nigra were evident.

In both cases, a striking finding was focal, massive occurrence of argyrophilic and AT8-positive fibrillary structures, with occasional intermingled coiled bodies, in the subcortical portion of the cerebral white matter, being more marked in the frontal lobe (Fig. 3a). The fibrillary structures often demonstrated a conspicuous astrocytic foot-like perivascular arrangement (Fig. 3b). RD3 and RD4 immunostaining revealed that the abnormally phosphorylated tau was purely 4-repeat tau (Fig. 3c). On the other hand, double-labeling immunostaining with AT8 and GFAP suggested that most of the subcortical AT8-positive fibrillary structures were of astrocytic origin (Fig. 3d, e). In this connection, in the affected subcortical white matter, there were no thorn-shaped astrocytes, which demonstrate prominent argyrophilic, and GFAP- and tau-positive cell bodies (no such astrocytes are evident in Fig. 3b–e) and are preferentially distributed in the mediobasal temporal lobe in elderly individuals [1, 13, 14, 22].

In both cases, the presence of ballooned neurons (Fig. 3f) and APs (Fig. 3g), with only a small number of pretangles/tangles, coiled bodies and threads, was also a feature in the affected cerebral cortex. There were no convincing TAs. Neuronal loss and gliosis in the cerebral cortex, if present, were only mild in degree. Fibrillary gliosis was evident in the cortico-white matter junctions. The neuropathological findings in cases 1 and 2 were summarized using the datasheet recommended by Dickson et al. [8] (Table 1). In addition, in both cases, the spinal cord segments (C7, T8, and L4) showed a moderate number of AT8-positive neuropil threads in the white matter, as well as AT8-positive neuropil threads and pretangles/tangles in the gray matter [15].

With the Gallyas–Brrak electron microscopic method, aggregates of silver particles were found almost exclusively in the cell processes that had no association with myelin sheaths (Fig. 4a). AT8 immunoelectron microscopy also revealed bundles of tau-positive straight and twisted tubules, about 18–22 nm in diameter, almost exclusively in the cell processes that had no association with myelin sheaths (Fig. 4b).

Additionally, in case 1, the spinal cord was markedly compressed at the level of the cervical segments due to cervical spondylosis. No Bunina bodies or ubiquitin-positive skeins characteristic of ALS [20] were found in the lower motor neurons, including the spinal anterior horn cells. In both cases, there were no histological or immunohistochemical features suggestive of associated Alzheimer's disease (neurofibrillary tangles: Braak–Braak stage = III; senile (amyloid) plaques: Braak–Braak stage = 0 [5]) or Parkinson's disease.

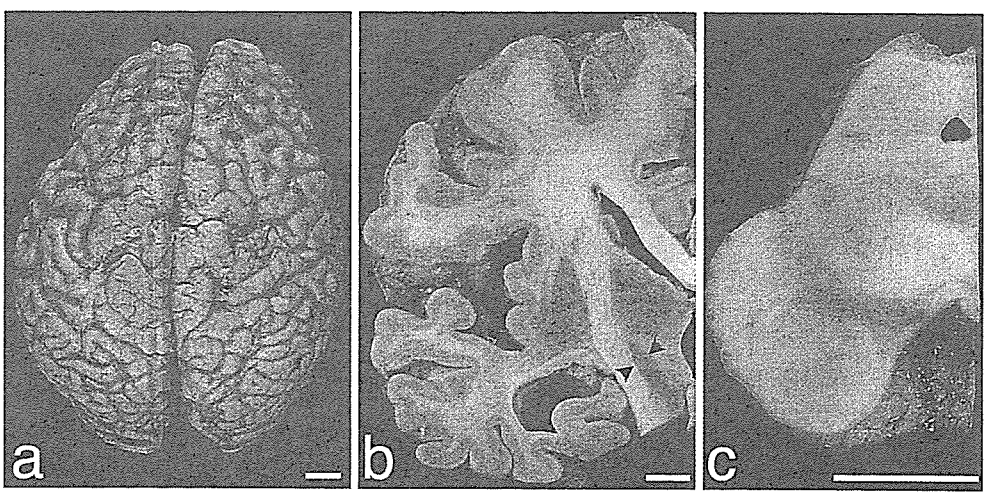

Fig. 2 Gross observation. **a** Symmetric, mild atrophy of the frontal lobe is evident. **b** Mild atrophy is also noted in the temporal lobe. The globus pallidus appears to be somewhat atrophic, whereas the subthamic nucleus appears normal (*arrowheads*). **c** Marked depigmentation is evident in the substantia nigra. **a–c** Case 1. *Bars* **a–c** 1 cm

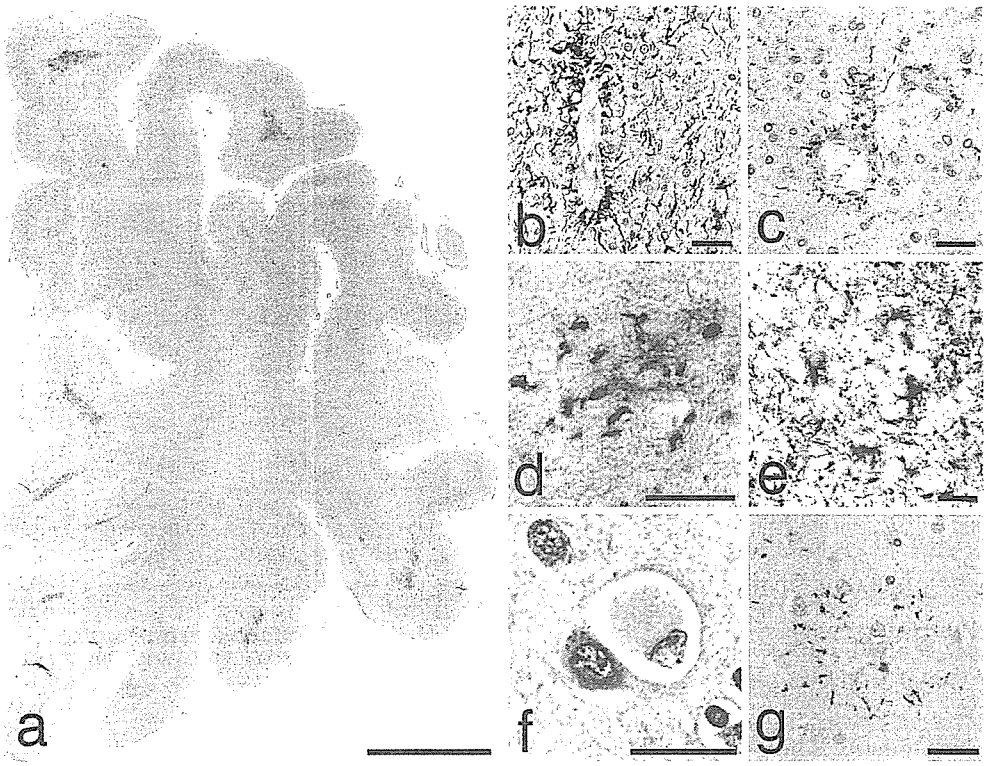

Fig. 3 Histological observation. **a** Coronal section of the frontal lobe, clearly showing focal, massive tau lesions in the subcortical regions of the white matter. Tau lesions are also noted in the cortex, but are not conspicuous. **b** Argyrophilic fibrillary structures are evident in the frontal subcortical white matter. Note a vessel surrounded by many argyrophilic structures of apparently astrocytic origin (vascular feet). **c** Tau-positive fibrillary structures, including those located in the perivascular region, are evident in the frontal subcortical white matter. **d** A tau-positive astrocytic plaque-like lesion in the frontal subcortical white matter. **e** Randomly arranged tau-positive structures, which surround astrocytic nuclei and perikarya, are evident in the frontal subcortical white matter. **f** A ballooned neuron in the frontal cortex. **g** A tau-positive astrocytic plaque in the temporal cortex. Note the scarcity of tau-positive pretangles/tangles and neuropil threads. **a**, **b**, **d**, **f**, **g** case 1; **c**, **e** case 2; **a** AT8; **b** Gallyas–Braak; **c**, **g** RD4; **d**, **e** Double-labeling immunostaining with AT8 (*brown*) and GFAP (*blue*); **f** hematoxylin–eosin. *Bars* **a** 1 cm; **b–g** 20 μm

Sarkosyl-insoluble tau and soluble tau, and the tau gene in case 2

Sarkosyl-insoluble tau extracted from the frontal and temporal lobes, and the midbrain of case 2 was resolved on immunoblots into two major bands of 68 and 64 kDa (Fig. 5). The low-molecular-mass fragments showed two closely related bands of approximately 37 kDa (Fig. 5). After dephosphorylation with alkaline phosphatase, the tau appeared as two major bands that aligned with the recombinant 4-repeat tau isoforms of 412 (4R, 1N) and 383 (4R, 0N) amino acids: the soluble tau from each case resolved into six bands in a pattern identical to that gleaned from the control brains (data not shown).

Sequence analysis of the tau gene showed no mutations (data not shown). All of the eight single nucleotide polymorphisms originally used to document the H1 and H2 haplotypes of the tau gene (exon 1 A5G, intron 2 C + 18T, intron 3 A + 9G, exon 9 A125G, exon 9 T209C, exon 9 G254A, intron 11 G + 34A, intorn 13 T + 34C) were homozygous for the H1 haplotype [4].

Discussion

In case 1, the disease was characterized by progressive dementia and amyotrophy. Although the spine MRI findings of evident cervical spondylosis and lumbar spine canal stenosis were available, ALS with dementia was considered to be a more probable clinical diagnosis. In case 2, the disease was characterized by progressive parkinsonism and dementia. The brain MRI findings of evident atrophy of the brainstem and cerebellum led us to the clinical diagnosis of MSA.

At autopsy, these clinical diagnoses were proved to be incorrect. In case 1, severe cervical spondylosis was considered to have been responsible for the amyotrophy. In case 2, there was no histopathological evidence

Table 1 Distribution and severity of major histological findings in our two patients

	Neuronal loss and gliosis		Ballooned neurons		Tau- or Gallyas-positive neurons		Tau- or Gallyas-positive glia		Tau- or Gallyas-positive threads	
Cases	1	2	1	2	1	2	1	2	1	2
Cerebral cortex										
Frontal	1	1	1	1	1	1	1	1	1	1
Motor (peri-Rolandic)	1	1	1	0	1	1	2	1	1	1
Cerebral white matter							1[a]	1[a]	1[a]	1[a]
Parietal	1	1	1	0	1	0	1	1	1	1
Temporal	1	1	1	1	1	1	2	1	1	1
Entorhinal	1	1	0	0	2[b]	1[b]	1	1	2[b]	1[b]
Subcortical areas										
Hippocampus	1	NA			2[b]	NA	0	NA	2[b]	NA
Amygdala	1	1	1	1	2	2	2	2	1	1
Basal nucleus of Meynert	1	1			2	1	0	0	1	1
Caudate & putamen	1	1			1	1	2	2	1	1
Globus pallidus	1	1			1	1	1	1	2	1
Internal capsule							1	1	1	1
Thalamus	1	1			1	1	1	1	1	1
Subthalamic nucleus	1	1			1	1	1	1	1	2
Brainstem										
Midbrain tectum (colliculi)	1	2			1	1	1	1	1	1
Red nucleus	NE	2			NE	1	NE	1	NE	1
Substantia nigra	2	2			2	2	1	1	2	2
Cerebral peduncle							1	1	1	1
Locus ceruleus	1	2			2	2	1	1	1	1
Pontine tegmentum	1	1			1	1	1	1	1	2
Pontine base	0	1			1	1	1	1	1	2
Fibers in pontine base							1	1	1	1
Inferior olivary nuclei	0	1			1	2	1	2	1	1
Cerebellum										
Dentate nucleus	1	2			1	1	1	1	1	2
Cerebellar white matter							1	2	1	1

The presence and severity of the histopathological features are represented as: 0 = none; 1 = minimal/mild; 2 = moderate/severe, *NA* not available due to fresh-to-recent ischemic lesions; *NE* not examined

[a]Excluding the subcortical tau lesions

[b]Alzheimer pathology (Braak–Braak stage = III, no senile plaques)

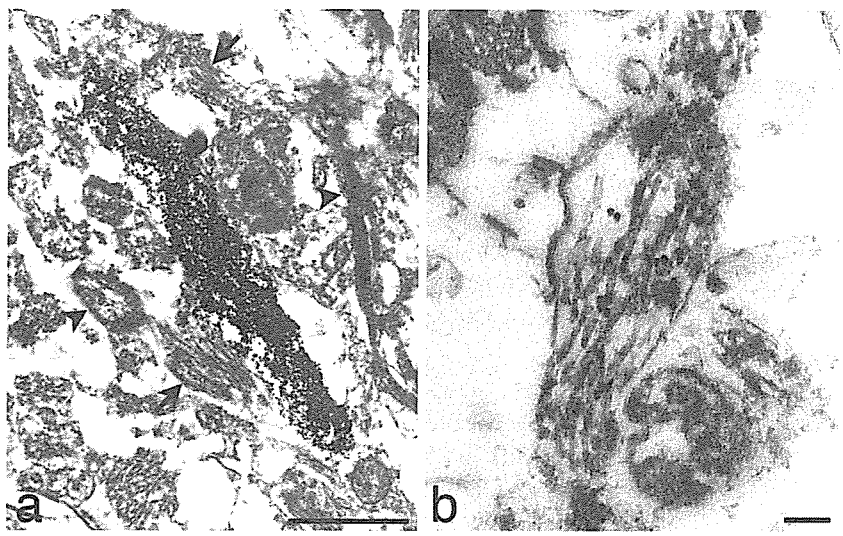

Fig. 4 Ultrastructural observation of the affected subcortical white matter. **a** Gallyas–Braak electron micrograph, showing an argyrophilic cell process. Note the neighboring astrocytic processes with bundles of glial filaments (*arrows*), as well as myelinated nerve fibers (*arrowheads*). **b** AT8 immunoelectron micrograph, showing a cell processes that contains a number of tau-positive straight and twisted tubules. **a** Case 2; **b** case 1. *Bars* **a** 1 μm; **b** 0.1 μm

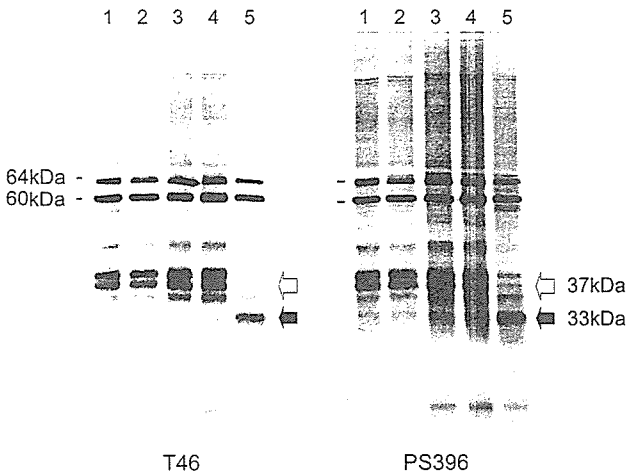

Fig. 5 Sarkosyl-insoluble tau from the temporal lobe (*lane 1*), frontal lobe (*lane 2*) and midbrain (*lane 3*) of case 2, as well as from the frontal lobe of a typical CBD case (*lane 4*) and typical PSP case (*lane 5*). The pattern of low-molecular-mass tau fragments in case 2 (*lanes 1–3*) is compatible with that of a typical CBD case, with two closely related prominent bands of 37 kDa (*lane 4*), while that of a typical PSP case reveals a prominent band of 33 kDa (*lane 5*). The antibody T46 recognizes the C-terminal region (amino acid residues 404–441) of human tau. The antibody PS396 recognizes the human tau phosphorylated at Serine 396, which is also located in the C-terminal region

of MSA. However, the autopsies also disclosed more essential neuropathology related to these patients' diseases. Apart from the topographical lesion severity, the neuropathological features of both cases were very similar to each other. Based purely on the presence of cortical ballooned neurons and APs, a neuropathological diagnosis of CBD seemed plausible in both cases [7, 8, 16, 24].

However, focal, massive occurrence of argyrophilic and tau-positive fibrillary lesions in the subcortical white matter is extremely unusual for brains affected by CBD. Moreover, double-labeling immunostaining, as well as Gallyas–Braak electron and AT8 immunoelectron microscopic studies revealed that both the subcortical argyrophilic and tau-positive fibrillary lesions occurred predominantly in the distal portion of the astrocytic processes; the fibrillary lesions were distinct from the so-called argyrophilic (tau-positive) threads, which occur mainly in the oligodendrocytic processes, more strictly, the inner and outer loops of the myelin sheaths [3, 12]. Therefore, also from a cytopathological viewpoint, argyrophilic and tau pathology affecting astrocytic processes, including the vascular feet, in the subcortical white matter is extremely unusual for CBD brains. It was also noteworthy that in both cases, argyrophilic and tau-positive neuronal (pretangles and tangles) and oligodendrocytic lesions (coiled bodies and neuropil threads in both the gray and white matter) were apparently small in number compared with those found in patients with CBD [7, 8, 24]. It is likely that the progressive dementia observed in both patients had been associated with the subcortical astrocytic tau pathology with fibrillary gliosis.

Interestingly, however, immunoblot analysis of insoluble tau performed in case 2 clearly demonstrated that the pattern was compatible with that obtained from brains affected by typical CBD pathology [2]. Gene analysis of tau also performed in case 2 showed no mutation in the gene, with the H1 haplotype; the finding of the H1 haplotype was consistent with the fact that the frequency of this haplotype in the Japanese population is essentially 100% [9]. Eventually, the neuropathological findings, together with the biochemical and genetic findings, strongly suggested that the present two patients had suffered from a unique, unusual variant of CBD.

We previously reported two Japanese cases of a possible CBD variant, in which co-occurrence of APs and TAs, and severe degeneration of the cerebral white matter were characteristic features; these two cases also showed cerebral tau biochemistry compatible with that found in CBD [23]. The present two cases, together with the two cases reported previously [23], suggest that although CBD is now considered to be a neuropathologically established tauopathy, it may include some variants showing unusual topographical and cellular tau pathologies.

In conclusion, we have described two sporadic cases of tauopathy showing unusual neuropathological features, for which a pathological diagnosis of a CBD variant, with focal massive tau accumulation in the subcortical white matter astrocytes, appears to be most preferable. However, whether or not this type of tau accumulation occurs only in association with neuropathological and/or biochemical features suggestive of CBD awaits further studies.

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Case Report

Co-localization of β-peptide and phosphorylated tau in astrocytes in a patient with corticobasal degeneration

Koichi Wakabayashi,[1] Fumiaki Mori,[1] Masato Hasegawa,[2] Tomomi Kusumi,[3] Ihoko Yoshimura,[4] Hitoshi Takahashi[5] and Sunao Kaneko[4]

[1]Department of Neuropathology, Institute of Brain Science, Hirosaki University School of Medicine, Hirosaki, [2]Department of Molecular Neurobiology, Tokyo Institute of Psychiatry, Tokyo Metropolitan Organization for Medical Research, Tokyo, Departments of [3]Pathology and [4]Neuropsychiatry, Hirosaki University School of Medicine, Hirosaki, and [5]Department of Pathology, Brain Research Institute, University of Niigata, Niigata, Japan

The co-localization of amyloid β (Aβ) and phosphorylated tau in astrocytes in a patient with corticobasal degeneration is described. At autopsy, the present case exhibited neuropathological findings compatible with those of corticobasal degeneration, including atrophy of the frontal and temporal lobes, neuronal loss and gliosis in the cortical and subcortical regions, and presence of cortical ballooned neurons and astrocytic plaques. Moreover, many senile plaques were found in the cerebral cortex. There were also clusters of Aβ-positive granules associated with astrocytic cytoplasm and processes in the subiculum and entorhinal cortex. In the entorhinal cortex, the Aβ-positive granules were occasionally co-localized with phosphorylated tau-positive fibrillary structures in the astrocytic cytoplasm. To our knowledge, this is the first demonstration of co-localization of Aβ and phosphorylated tau in astrocytes. This phenomenon implies that phagocytosis of Aβ coincides with production of phosphorylated tau in the same reactive astrocytes.

Key words: β-peptide, corticobasal degeneration, phosphorylated tau, thorn-shaped astrocyte.

INTRODUCTION

Phosphorylated tau and amyloid β-peptide (Aβ) are the major components of NFT and senile plaques, respectively, which are present abundantly in the brains of patients with Alzheimer's disease (AD). Tau proteins are also the constituents of neuronal and glial fibrillary alterations in various neurodegenerative disorders referred to as "tauopathies"; abnormal accumulations of phosphorylated tau proteins are present in both astrocytes and oligodendrocytes.[1,2] Moreover, Aβ-containing astrocytes and microglias have been reported to occur in AD and the aged human brain.[3–7] In this paper, we report the co-localization of Aβ and phosphorylated tau in astrocytes of the entorhinal cortex in a patient with corticobasal degeneration (CBD).

CLINICAL SUMMARY

The patient, a 63-year-old woman, noted that her speech was becoming progressively slower and more labored, although she continued a full range of activities. At age 64, her speech was slow and grammatically incorrect, and she had difficulty finding words. However, she was still able to read and write, and there were no focal neurological signs. MRI revealed hyper-signal intensity on T2-weighted images in the bilateral frontal and parietal white matter. Thereafter, she became forgetful and her verbal speech deteriorated markedly. By age 67, she had lost all meaningful verbal expression, and naming was severely impaired. At age 69, she was hospitalized because of frequent wandering and abnormal behavior. Examination revealed mild bradykinesia, a mask-like face and stereotypy. The grasping reflex was positive on both sides. In the following year, she became bedridden and totally mute. A further MRI examination revealed progression of the cerebral atrophy, especially of the frontal and temporal lobes, and expansion of hyper-signal intensity on T2-weighted

Correspondence: Koichi Wakabayashi, MD, Department of Neuropathology, Institute of Brain Science, Hirosaki University School of Medicine, 5 Zaifu-cho, Hirosaki 036-8562, Japan. Email: koichi@cc.hirosaki-u.ac.jp

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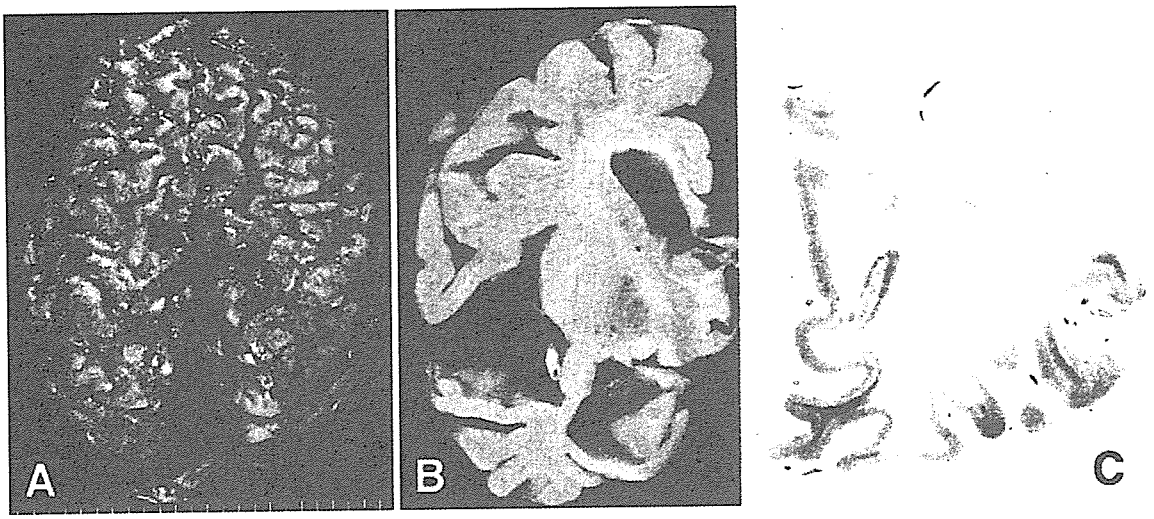

Fig. 1 (A) Gyral atrophy is evident in the convex areas of the frontal lobes. (B) Brownish-tan discoloration is evident in the globus pallidus. (C) Severe myelin pallor is evident in the frontal white matter. KB stain.

images in the cerebral white matter. The patient died at the age of 75 years, 12 years after the onset of the symptoms. There was no family history of neurological or psychiatric illness.

PATHOLOGICAL FINDINGS

Postmortem examination revealed a severely atrophic brain weighing 785 g (Fig. 1A). Sections of the cerebrum and brainstem showed marked dilatation of the lateral ventricles, atrophy of the globus pallidus (Fig. 1B) and marked depigmentation of the substantia nigra. For light microscopy, 4-μm-thick, formalin-fixed, paraffin-embedded sections were stained with HE or by the KB or Gallyas–Braak method. Other sections were immunostained using a polyclonal antibody against human Aβ (1–42) (IBL, Fujioka, Japan; 1:100), or monoclonal antibodies against phosphorylation-dependent epitopes of tau protein (AT8; Innogenetics, Ghent, Belgium; 1:200) or GFAP (IBL; 1:50). Selected sections were double-immunolabeled with combinations of anti-Aβ and either anti-GFAP or AT8. Vibratome sections (50 μm) from the temporal cortex and midbrain were processed for electron microscopy.

Histological examination revealed marked neuronal loss, gliosis, spongy degeneration and occurrence of ballooned neurons in the cerebral cortex (Fig. 2A), predominantly in the frontal, cingulate and temporal cortices. The frontal and temporal white matter exhibited severe loss of myelin and axons with tissue rarefaction and fibrillary gliosis (Fig. 1C). There was severe neuronal loss with gliosis in the globus pallidus, substantia nigra and locus ceruleus. Neurons were also depleted in the neostriatum, thalamus and cerebellar dentate nucleus.

Although typical NFT were almost totally restricted to the hippocampus (Braak stage II),[8] staining with the Gallyas–Braak method and AT8-immunostaining revealed widespread accumulation of phosphorylated tau in the neuronal and glial cytoplasm and their processes in the cerebral cortex and white matter, as well as in the basal ganglia and brainstem (Fig. 2B–F). Many pretangles were observed in the histologically affected CNS regions. Coiled bodies were observed widely in the brain, whereas astrocytic plaques were restricted to the affected cerebral cortex and striatum. Electron microscopy showed that neuronal and glial tau-positive structures comprised exclusively bundles of straight tubules about 15 nm in diameter (Fig. 2G).

Moreover, many senile plaques, chiefly the diffuse and primitive forms, were found in the cerebral cortex (Braak stage C) (Fig. 2H).[8] There were also clusters of Aβ-positive granules associated with oval and vesicular nuclei in the subiculum and entorhinal cortex (Fig. 2I). Double immunostaining showed that these Aβ-positive granules were located in astrocytes (Fig. 2J), and also in the astrocytic end-feet around blood vessels (Fig. 2K). In the entorhinal cortex, the Aβ-positive granules were occasionally co-localized with phosphorylated tau-positive fibrillary structures in the cytoplasm of astrocytes (Fig. 2L).

Sarkosyl-insoluble tau and soluble tau were extracted from the frontal lobe of the present patient as well as from the frontal lobe of a typical AD case and a control case and dephosphorylation of insoluble tau was performed as described previously.[9] Tau proteins before and after dephosphorylation were run on a 10% sodium dodecyl

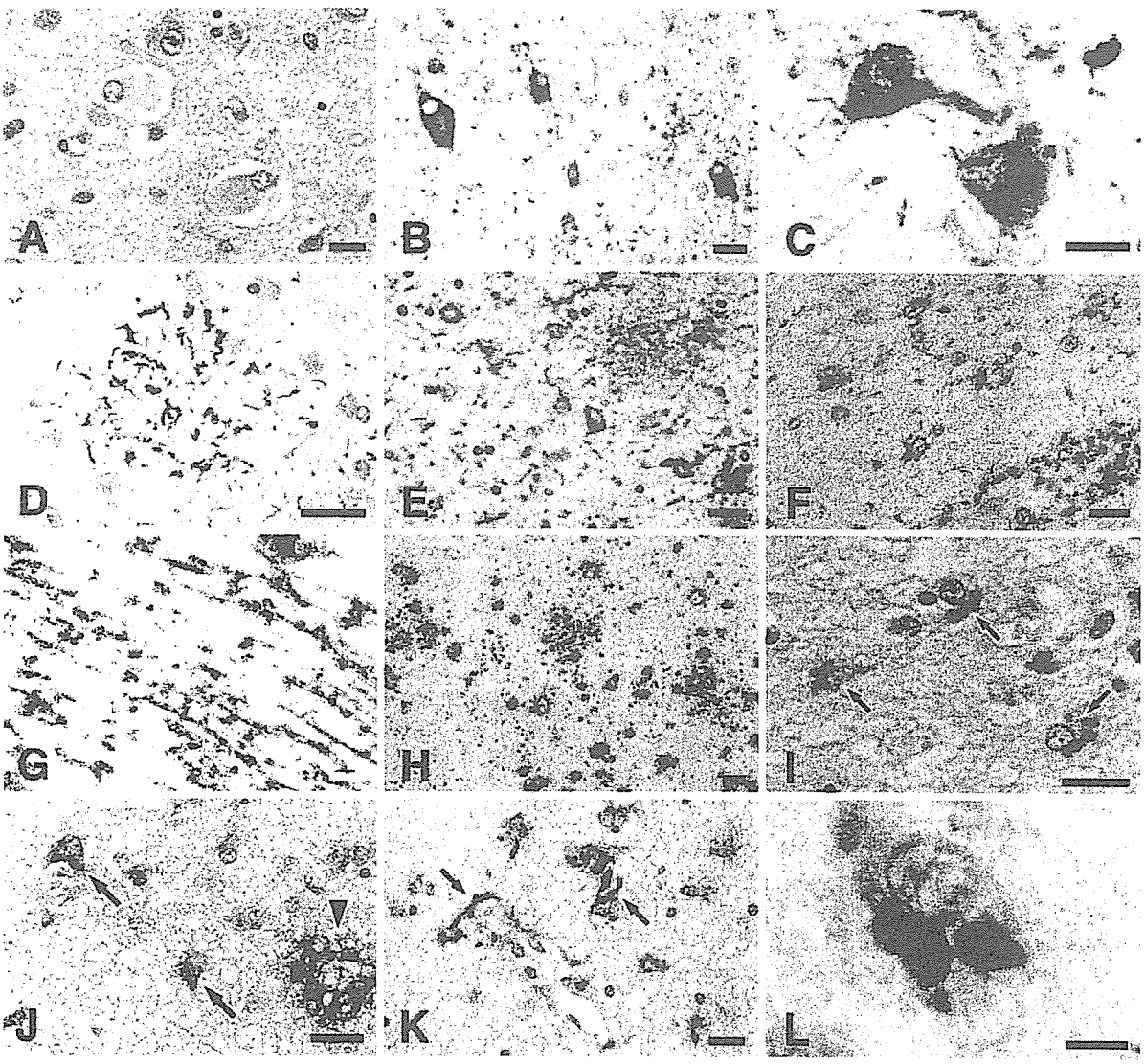

Fig. 2 (A) Ballooned neurons in the cingulate cortex. (B,C) Accumulation of phosphorylated tau in the neuronal cytoplasm and processes in the frontal cortex (B) and red nucleus (C). (D) Astrocytic plaques in the frontal cortex. (E) Coiled bodies in the frontal white matter. (F) Thorn-shaped astrocytes in the temporal white matter. Tau-positive, astrocytic end-feet are also evident (lower right). (G) Ultrastructure of neurofibrillary alterations in the red nucleus showing bundles of 15-nm-wide straight tubules. (H) Many senile plaques in the temporal cortex. (I) Clusters of Aβ-positive granules in the subiculum (arrows). (J,K) Double-immunostaining for Aβ (dark blue) and for GFAP (brown) showing Aβ granules in the astrocytic cytoplasm (J, arrows) and end-feet around the blood vessels (K, arrows). (J) Arrowhead indicates a primitive plaque. (L) Double-immunostaining for tau (dark blue) and for Aβ (brown) showing co-localization of Aβ granules and tau-positive fibrillary structures in the astrocytic cytoplasm. (A) HE; (B,C,E,F) AT8-immunostain; (D) Gallyas–Braak; (H,I) Aβ-immunostain.

sulfate-polyacrylamide gel, transferred to a polyvinylidene difluoride membrane, and probed with a phosphorylation-independent anti-tau antibody (HT7; Innogenetics; 1:5000). Sarkosyl-insoluble tau extracted from the frontal lobe was resolved on immunoblots into two major bands of 68 and 64 kDa and a minor band of 72 kDa (Fig. 3). After dephosphorylation with alkaline phosphatase, the tau appeared as two major bands that aligned with the recombinant four-repeat tau isoforms of 412 (4R, 29 N) and 383 (4R, 0 N) amino acids: the soluble tau resolved into six bands in a pattern identical to that gleaned from the control brain (Fig. 3).

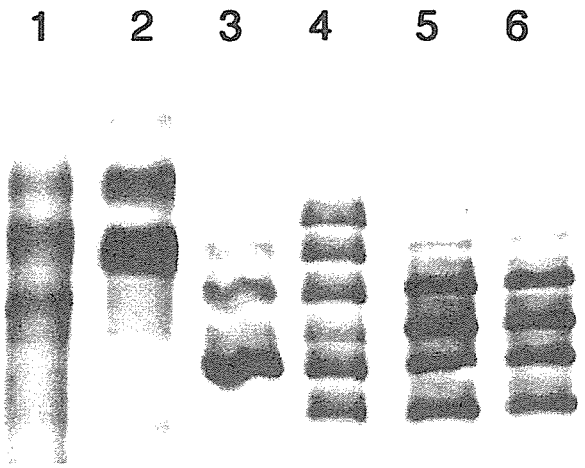

Fig. 3 Sarkosyl-insoluble tau from the frontal lobe of Alzheimer's disease case (lane 1) and the present case (lane 2). In the present case, dephosphorylation reveals two major and some minor bands (lane 3). The major bands align with 29 N4Rtau and 0 N4Rtau isoforms. Lane 4 shows all six recombinant tau isoforms. Soluble tau from the frontal lobe of control case (lane 5) and the present case (lane 6). The antibody HT7 recognizes amino acid residues 159–163 of human tau.

DISCUSSION

Our patient initially presented with progressive aphasia and subsequently developed dementia. The pathological features of our patient were: (i) neuronal loss with gliosis in the cerebral cortex and subcortical nuclei, especially the globus pallidus and substantia nigra; (ii) occurrence of ballooned neurons; and (iii) widespread accumulation of abnormally phosphorylated tau in both neuronal and glial cells (astrocytic plaques and coiled bodies). These histopathological features are identical to those of CBD,[10–15] and some CBD patients have been reported to present with primary progressive aphasia.[16,17] Although there were many senile plaques, the majority were diffuse and primitive forms, whereas the mature neuritic plaques typical of AD were scarcely evident. Biochemical analysis of the brain revealed selective accumulation of four-repeat tau, a feature of CBD.[18,19] We therefore considered that this patient had suffered from CBD.

In our patient, Aβ-positive granules were found in the cytoplasm and processes of astrocytes. Consistent with previous studies, these astrocytes were located preferentially in the deep layer of the entorhinal cortex.[6] Funato *et al.* reported diffuse plaques associated with Aβ-positive granules in 19 of 102 non-demented subjects over 60 years old (18.6%) and two of 11 patients with AD (18.1%).[4] Aβ in astrocytes could be either produced internally or taken up from an outside source. Because expression of the amyloid precursor protein in astrocytes is known to be extremely low,[20–22] internal production is unlikely to be a major source of accumulated Aβ. By contrast, internalization of Aβ from an exogenous source is more likely and could occur via receptor-mediated endocytosis and/or phagocytosis.[23] Indeed, *in vivo* and *in vitro* studies have confirmed that astrocytes have phagocytic activity.[24–26] Previous ultrastructural studies have shown that the Aβ-positive granules are located in lipofuscin-like granules and appear in lysosomal structures.[4,5] Thus, it appears that astrocytes engulf Aβ and degrade it in lysosomes in some parts of the aged human brain.

The morphology and distribution of tau-positive astrocytes observed in our patient are identical to those of thorn-shaped astrocytes that are known to appear in a variety of disorders associated with NFT.[27,28] However, there is no relationship between the severity of NFT and the incidence of this type of astrocyte, because it is rather scant in the brains of patients with AD. Ultrastructurally, thorn-shaped astrocytes contain bundles of 15-nm straight tubules,[27] suggesting that these abnormal fibrils are formed by the glial cells themselves. Several investigators have suggested that thorn-shaped astrocytes may be reactive in nature.[27,29] In the present case, thorn-shaped astrocytes in the entorhinal cortex occasionally contained Aβ-positive granules. To our knowledge, this is the first demonstration of co-localization of Aβ and phosphorylated tau in astrocytes. This phenomenon implies that phagocytosis of Aβ coincides with production of phosphorylated tau in the same reactive astrocytes. As described above, the occurrence of Aβ-containing astrocytes and thorn-shaped astrocytes is not uncommon. However, the concurrence of both changes in the same astrocytes has not been reported hitherto. This can be explained by the fact that the preferential sites of these alterations do not overlap each other; Aβ-containing astrocytes are located most commonly in the deep layer of the entorhinal cortex as well as in layer III of the cerebral neocortex,[4,6] whereas thorn-shaped astrocytes are generally confined to the subpial and subependymal regions of the gray and white matter and are found only occasionally in the deep cortical layer.[27,28] In the present case, astrocytes with coexistent Aβ and phosphorylated tau were virtually restricted to the deep layer of the entorhinal cortex. Although the occurrence of Aβ-containing microglias and phosphorylated tau-bearing oligodendrocytes has been described, co-localization of Aβ and phosphorylated tau in these glial cells has not been reported.

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