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Signature Tau Neuropathology in Gray and White Matter of Corticobasal Degeneration

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Corticobasal degeneration (CBD) is an adult-onset progressive neurodegenerative disorder characterized by L-dopa-resistant rigidity, focal cortical deficits, and variable dementia. The neuropathological hallmark of CBD is the deposition of filamentous inclusions in neurons and glia composed of hyperphosphorylated tau with only four microtubule-binding repeats (4R-tau). To characterize the regional burden of tau pathology in CBD, we studied 12 brains with the neuropathological diagnosis of CBD using biochemical and histochemical techniques. Eleven brain regions were evaluated including gray and white matter from frontal, parietal, temporal, and occipital lobes and cerebellum as well as basal ganglia. Although the distribution of tau pathology was variable, neuropathological and biochemical data showed a similar burden of tau abnormalities in frontal, temporal, and parietal lobes and basal ganglia of both hemispheres. This included abundant, sarkosyl-insoluble 4R-tau in both gray and white matter of two or more of these cortical regions and basal ganglia, and to a lesser extent, cerebellar white matter. The insoluble tau pathology in gray and white matter showed overlapping but distinct phosphorylated epitopes suggesting cell-type and subcellular localization (ie, cell bodies *versus* cell processes)-specific differences in tau phosphorylation. In contrast, soluble tau was composed of normal 4R/3R-tau ratios indicating no gross abnormality in tau splicing. Thus, although clinically heterogeneous, CBD is a distinct lobar and basal ganglionic tauopathy with selective aggregation of 4R-tau. *(Am J Pathol 2002, 160:2045–2053)*

Corticobasal degeneration (CBD) was first described in 1968 as a progressive neurological disorder clinically characterized by abnormalities in posture and motor function with relatively intact mental faculties.[1] Neuropathological examination revealed atrophy of the frontoparietal cortex with neuronal loss and gliosis in affected cortices and the substantia nigra as well as distinctive, swollen, pale neurons in the cortex. Hence, the term corticodentatonigral degeneration with neuronal achromasia for a disorder later known as CBD. Subsequent reports emphasized the distinct motor features of CBD with relatively intact cognition.[2–6] However, a number of reports described clinical heterogeneity in patients with pathologically confirmed CBD including presentations similar to Alzheimer's disease (AD), frontotemporal dementia, primary progressive aphasia, and progressive supranuclear palsy (PSP).[7–17]

The contemporary neuropathology of CBD includes prominent atrophy of parasagittal cortex particularly in peri-Rolandic regions, as well as depigmentation of the substantia nigra.[18] In affected regions, there is neuronal loss, gliosis, and prominent glial and neuronal intracytoplasmic filamentous tau-immunoreactive pathology.[19,20] Achromatic ballooned neurons, that are most numerous in cortical and limbic regions, are strongly immunoreactive for phosphorylated neurofilaments and αB-crystallin but variably positive for tau.[21,22] The glial tau pathology consists of characteristic astrocytic plaques as well as numerous tau-immunoreactive inclusions in gray and white matter in astrocytes and oligodendrocytes (coiled bodies).[23,24] Perhaps the most striking feature of CBD is the extensive accumulation of tau-immunoreactive cell processes throughout both the gray and white matter.[25] Unlike the neuropil threads of AD, the majority of threads do not stain with antibodies to neurofilament suggesting many are localized in glia.[25]

The pathological filamentous inclusions in CBD are composed primarily of abnormally phosphorylated tau similar to many other sporadic and familial neurodegenerative diseases such as AD, PSP, Pick's disease (PiD), and frontotemporal dementia with parkinsonism linked to

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Table 1. Clinical Characteristics of Patients with Pathologically Proven CBD

Patient	Age at death, years	Gender	Duration, years	PMI, hours	Clinical presentation	Clinical diagnosis
1	77	M	5	18	Progressive memory loss associated with speech difficulties	AD
2	56	M	3	NA	Progressive dementia, depression, and paranoia	Unclassified
3	64	F	3	14	Asymmetric motor dysfunction	CBD
4	68	M	6	13	Hypersexuality, increasing gregariousness, and marked irritability	FTD
5	76	F	3	12	Memory loss, frequent falls	CBD
6	61	F	7	15	Loss of manual dexterity, right hand, and slurring of speech	CBD
7	71	M	6	18	Visual agnosia, memory loss	Atypical AD
8	70	F	8	17	Altered speech patterns, anhedonia, decreased energy levels with increased somnolence	FTD
9	73	F	8	NA	Left upper extremity stiffness, numbness and twisting	CBD
10	71	F	6	NA	Decreased manual dexterity of hands, progressive slowing of motor functions	CBD
11	59	F	6	NA	Shaking and pains in hands, parkinsonism	CBD
12	71	F	7	6	Parkinsonism, memory loss	PSP

chromosome 17 (FTDP-17), collectively referred to as tauopathies.[26–28] Tau is a phosphoprotein that regulates the assembly and stability of microtubules.[29,30] In the central nervous system (CNS), six tau isoforms are produced by alternative splicing.[31,32] Alternative splicing of exons 2 and 3 results in the insertion of 0, 29, or 58 amino acids near the amino-terminus. In the carboxy-terminal half of the molecule, alternative splicing of exon 10 results in either three or four microtubule-binding motifs (3R-tau and 4R-tau, respectively).[31,32] In AD, the filamentous tau aggregates are composed of all six tau isoforms, but in CBD and PSP, the aggregates are composed predominantly of 4R-tau whereas in PiD, they are composed of 3R-tau.[26–28]

In this study, we analyzed tau pathology in the brains of 12 patients with pathologically confirmed CBD. We demonstrate extensive heterogeneity in tau abnormalities in CBD; however, there is a distinct phenotype characterized by the widespread aggregation of 4R-tau throughout the brain. The extent of tau pathology in gray and white matter detected by Western blot analysis correlated with that detected by immunohistochemistry (IHC).

Materials and Methods

Patients

All patients met histopathological criteria for the diagnosis of CBD assessed independently by at least two neuropathologists (MSF, SSMC, CB, and JQT).[18,19] Diagnostic criteria emphasized a cortical and subcortical distribution of tau pathology in both gray and white matter including neuronal and glial tau-positive inclusions and extensive thread pathology.[19] Although present in most cases, astrocytic plaques and ballooned neurons were not required for the diagnosis of CBD. The age, gender, and clinical diagnosis of these patients are given in Table 1. Patients 2 and 4 have already been presented elsewhere.[9] Control AD brain tissue was obtained through the University of Pennsylvania Alzheimer's Disease Center.

Histochemistry and IHC

Tissue obtained at the time of autopsy was fixed in either neutral-buffered formalin or 70% ethanol in 150 mmol/L NaCl, pH 7.4, paraffin-embedded, and cut into 6- to 10-μm-thick sections. Sections were stained with hematoxylin and eosin, Thioflavin S, and Gallyas silver stains. IHC was performed on sections of neocortex, basal ganglia, and cerebellum using a panel of antibodies to tau as well as α-synuclein and phosphorylated neurofilament (Table 2). The avidin-biotin-peroxidase method with 3,3′-diaminobenzidine for color development was used for all immunostaining. Tau pathology in both gray and white matter was assessed as absent, mild, moderate, or severe.

Tau Preparations and Western Blot Analysis

Fresh, frozen brain tissue from four neocortical brain regions (frontal, parietal, temporal, and occipital lobes), basal ganglia, and cerebellum including deep white matter from each case (where available) were used for biochemical analysis. Tissue was obtained from the contralateral hemisphere from that used for histochemical analysis. Gray matter and white matter were dissected from each brain region (except basal ganglia) and soluble and insoluble tau proteins were extracted as described previously.[33] Soluble and insoluble tau fractions were resuspended in 10 mmol/L Tris, 1 mmol/L ethylenediaminetetraacetic acid, pH 7.6, at concentrations of 0.5 and 0.1 ml/g of tissue, respectively. Where indicated, tau was dephosphorylated by treatment with *Escherichia coli* alkaline phosphatase (Sigma, St. Louis, MO) at 67°C for 1 hour. For Western blot analysis nitrocellulose replicas were prepared from 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) slab gels containing either the soluble or insoluble protein

Table 2. Antibodies Used to Characterize Pathology

Antibody	Source	Specificity	Recognition site	Reference
17026	Rabbit	PI tau*	Recombinant tau	50
Tau 14	Mouse	PI tau	141–178	51
AT270	Mouse	P tau†	Thr 181	52
AT8	Mouse	P tau	Ser 202/Thr 205	53
PHF6	Mouse	P tau	Thr 231	54
12E8	Mouse	P tau	Ser 262	55
PHF1	Mouse	P tau	Ser 396/Ser 404	56
Tau 46	Mouse	PI tau	404–441	51
RMO24	Mouse	P-NFH	Multiphosphorylation repeats in NFH	57
LB509	Mouse	α-synuclein	115–122	58

*PI, phosphorylation independent.
†P, phosphorylated.

samples and probed with antibodies for tau as indicated. Monoclonal and polyclonal antibodies were detected with horseradish peroxidase-conjugated anti-mouse and anti-rabbit IgG, respectively (Santa Cruz Biotechnologies, Santa Cruz, CA). Immunoreactive proteins were revealed using the enhanced chemiluminescence (NEN Life Science, Boston, MA) and/or 3,3′-diaminobenzidine detection systems. Relative quantities of insoluble tau pathology were assessed as absent, mild, moderate, or severe.

Results

Topographic Distribution of Tau Pathology in CBD Patients

The neuropathological diagnosis of CBD was confirmed in all 12 cases using criteria that emphasize a cortical and subcortical distribution of tau pathology in both gray and white matter.[18,19] This includes neuronal and glial tau-positive inclusions including extensive thread pathology and astrocytic plaques as well as phosphorylated neurofilament-positive ballooned neurons (Figures 1 and 2). In two cases (patients 1 and 2), there was hippocampal sclerosis consistent with previous ischemic/hypoxic injury. None of the 12 cases fulfilled criteria for other neurodegenerative disorders including AD, Lewy body disorders, PSP, and PiD. The main findings on immunohistochemical analysis for tau are summarized in Table 3. In grading the severity of regional involvement, emphasis was placed on the thread pathology in both the gray and white matter using at least two antibodies specific for tau as well as Gallyas silver stains because this is the most prominent abnormality in CBD (Figure 2). There was extensive, but highly variable, tau pathology in both the gray and white matter throughout all brain regions of

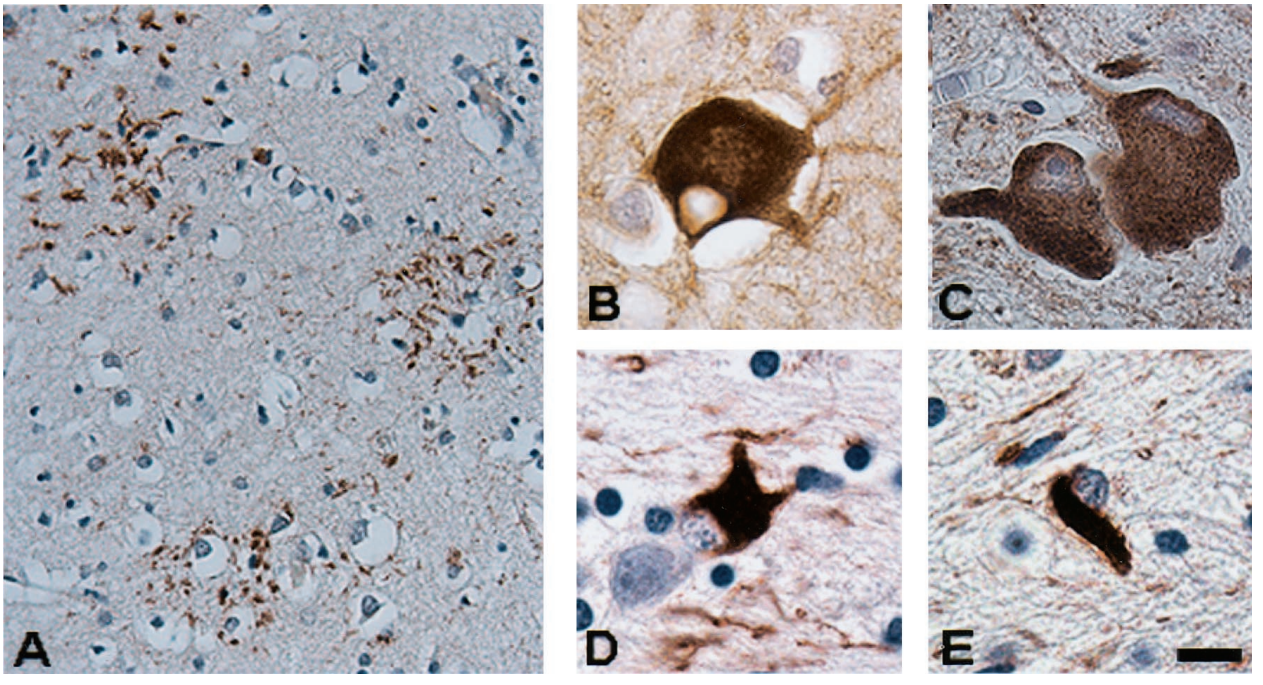

Figure 1. Characteristic brain lesions in CBD. **A:** Astrocytic plaque in neocortex (tau immunostain, PHF1). **B:** Balloon neuron in neocortex (neurofilament immunostain, RMO24). **C:** Neuronal cytoplasmic inclusions in nucleus basalis (tau immunostain, PHF1). **D:** Astrocytic cytoplasmic inclusion in neocortex (tau immunostain, PHF1). **E:** Oligodendroglial inclusion (coiled body) in subcortical white matter (tau immunostain, PHF1). Scale bars: 30 μm (**A**); 10 μm (**B–E**).

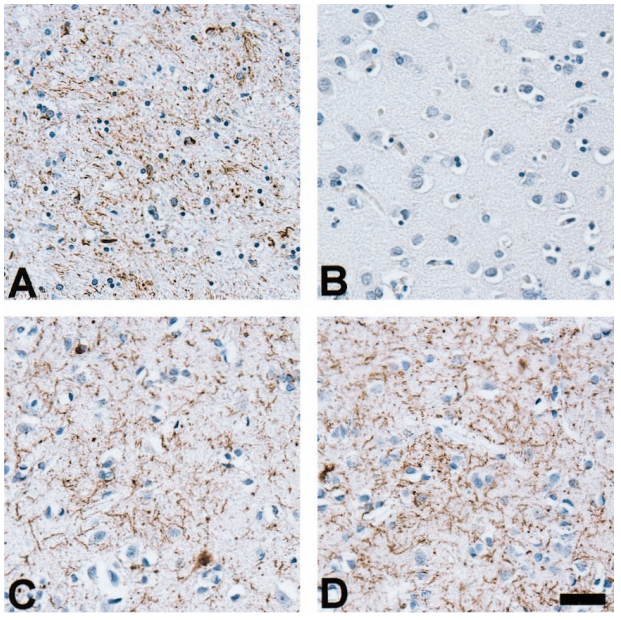

Figure 2. Topographic distribution of tau-immunoreactive pathology shows regional variability. **A:** Marked thread pathology in the basal ganglia of patient 10. **B:** In contrast, the occipital lobe of patient 10 is virtually devoid of tau pathology. **C** and **D:** Thread pathology in both the frontal lobe and occipital lobe of patient 7 who presented with a visual agnosia. Tau immunostains, PHF1. Scale bar, 80 μm.

each patient except for occipital lobes and cerebellar gray matter (Figure 2 and Table 3). The basal ganglia and the frontal lobes were moderately to severely affected in all patients except patients 1 (basal ganglia) and 5 (frontal lobe). Both the gray and white matter of the temporal and parietal lobes as well as the cerebellar white matter showed variable involvement, ranging from mild to severe pathology, but the extent of white matter tau pathology was at least equivalent to that in gray matter. The topographic distribution of tau-immunoreactive pathology only loosely correlated with the clinical presentation and/or diagnosis of the patient (Tables 1 and 3). These results are not surprising because focal involvement of the neocortex may account for specific clinical phenotypes and in this analysis, we sampled specific cortical regions to standardize comparison between patients.[6,9,17,34,35]

Biochemical Characterization and Topographic Distribution of Sarkosyl-Insoluble Tau in CBD

To further analyze tau pathology in CBD, we performed Western blot analysis of sarkosyl-insoluble tau from multiple neocortical regions as well as basal ganglia and cerebellum from the hemisphere contralateral to that used for histochemical and IHC analysis. In all patients, insoluble tau in both gray and white matter was composed of two major protein bands of ~64 and 68 kd (Figures 3 and 4). On dephosphorylation, these major protein bands co-migrated with 4R-tau isoforms but 3R-tau with one amino terminal insert could occasionally be detected in some of the cases (Figure 3, C and D). The presence of 4R-tau was confirmed with an antibody specific for exon 10 that encodes for the alternatively spliced microtubule-binding domain that distinguishes 4R-tau from 3R-tau (data not shown). In contrast, AD tau pathology is localized predominantly within gray matter and is composed of both 4R- and 3R-tau isoforms (Figure 3, C and D).

Similar to IHC, a Western blot analysis of the topographic pattern of insoluble tau showed extensive tau pathology in both gray and white matter throughout the brain of all patients (Figure 4 and Table 4). The basal ganglia consistently demonstrate robust tau pathology. Also consistent with IHC, the neocortex showed mild to marked pathology in the frontal, parietal, and temporal lobes of all patients. In contrast, the occipital lobe and cerebellum were the only brain regions examined wherein tau pathology occasionally was absent. The biochemical distribution of insoluble tau pathology loosely correlated with the distribution of tau pathology revealed by IHC with a Spearman's rank order correlation coefficient equal to 0.37 [$S(38) = 0.37$; $P < 0.02$]. The differences most likely reflect sampling because tau pathology in CBD is often focal and asymmetric.[18] However, the present findings of tau pathology in both

Table 3. Tau-Immunoreactive Pathology in CBD, IHC

	Frontal lobe		Temporal lobe		Parietal lobe		Occipital lobe		Basal ganglia		Cerebellum	
Patient	Grey matter	White matter	Grey matter	White matter	Grey matter	White matter	Grey matter	White matter	Striatum	Globus pallidus	Folia	White matter
1	2	3	NA	NA	2	1	0	0	1	1	0	1
2	1	3	1	1	NA	NA	NA	NA	3	3	0	2
3	3	3	1	0	2	2	NA	NA	2	3	0	2
4	3	3	2	2	2	2	NA	NA	3	3	0	2
5	1	1	1	1	1	1	0	0	2	2	0	NA
6	3	3	2	2	2	2	0	0	3	3	0	NA
7	2	2	2	3	2	3	2	1	3	NA	0	NA
8	2	2	2	2	2	1	0	0	NA	3	0	1
9	3	3	2	2	1	2	0	1	3	3	0	2
10	2	2	1	1	1	1	0	0	3	3	0	1
11	2	3	1	1	1	2	1	1	3	3	0	2
12	3	3	2	2	3	3	0	0	3	3	0	2

Frontal lobe, mid-frontal gyrus; temporal lobe, superior temporal gyrus; parietal lobe, inferior parietal lobule; occipital lobe, calcarine cortex.
0, absent; 1, mild density; 2, moderate density; 3, high density; NA, not available.

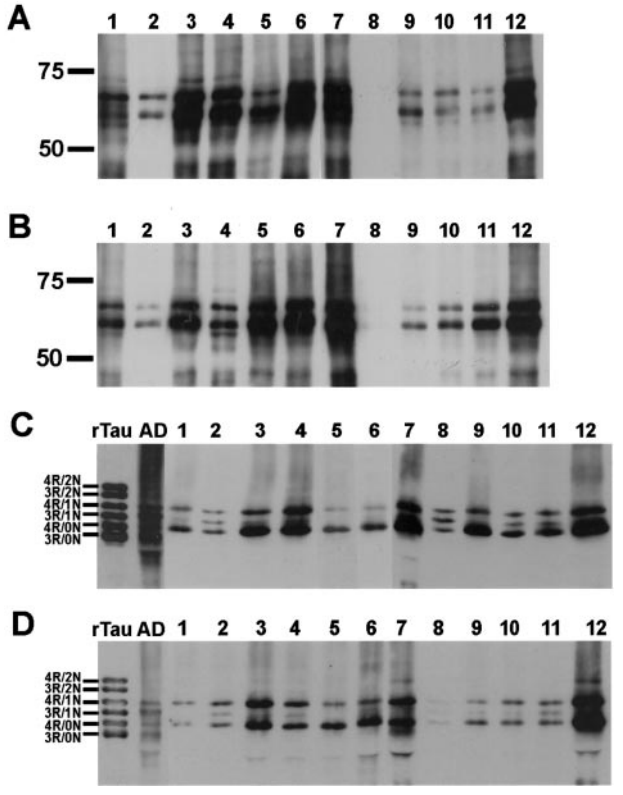

Figure 3. Western blot analysis of insoluble tau in gray and white matter of the parietal lobe of CBD brains shows marked interpatient variability. Insoluble tau fractions extracted from gray (**A**) and white (**B**) matter of the parietal lobe of 12 CBD brains were resolved by SDS-PAGE and immunoblotted with PHF1. The insoluble tau protein is composed predominantly of two major proteins of 64 and 68 kd. Aliquots of the insoluble fractions were dephosphorylated with *E. coli* alkaline phosphatase, resolved by SDS-PAGE, and immunoblotted with T14 and T46 that recognize phosphorylation-independent tau epitopes on the amino- and carboxy terminus, respectively. On dephosphorylation, the insoluble tau fractions from gray (**C**) and white (**D**) matter co-migrate with 4R-tau; 3R-tau with one amino terminal insert could occasionally be detected in some of the cases. In contrast, in AD, dephosphorylated, insoluble tau fractions co-migrate with both 4R-tau and 3R-tau isoforms. Prolonged exposure of the immunoblots revealed at least mild tau pathology in all samples as reflected in Table 4. Recombinant tau isoforms (rTau) are indicated to the **left** of **C** and **D**. Molecular weight standards are as indicated to the **left** of each panel here and in Figures 4 and 5.

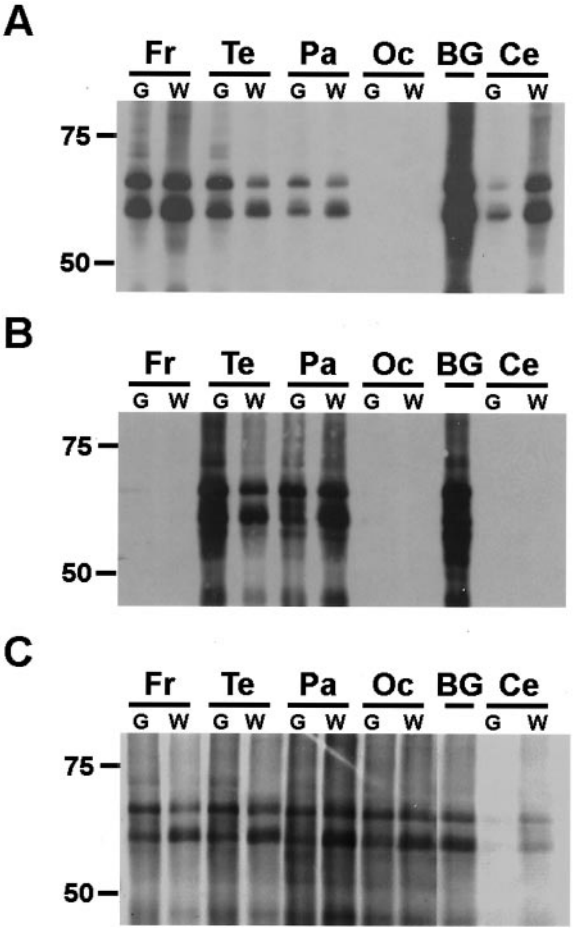

Figure 4. Western blot analysis of insoluble tau in CBD shows marked regional variability. Insoluble tau fractions from the brain regions indicated were resolved by SDS-PAGE and immunoblotted with PHF1. The insoluble tau protein from all brain regions is composed predominantly of two major proteins of 64 and 68 kd. Patient 2 (**A**), who presented clinically with an unclassified dementia, shows the most severe pathology in the frontal and temporal lobes. Patient 1 (**B**), who was diagnosed clinically with AD, shows the most severe pathology in the parietal and temporal lobes. Prolonged exposure of the immunoblot revealed mild tau pathology in frontal and occipital lobes as well as cerebellum as reflected in Table 4. In contrast, patient 4 (**C**), who was diagnosed clinically with frontotemporal dementia, shows moderate pathology in all neocortical regions. Fr, frontal lobe; Te, temporal lobe; Pa, parietal lobe; Oc, occipital lobe; BG, basal ganglia; Ce, cerebellum; G, gray matter; W, white matter.

cerebral hemispheres argue that CBD is a bilateral disorder with an asymmetric distribution of the severity of the tau pathology that may account for the specific clinical phenotypes.

Gray and White Matter Demonstrate Qualitative Differences in Tau Phosphorylation

To further characterize the biochemical properties of insoluble tau in gray and white matter of CBD patients, we performed Western blot analysis with a panel of antibodies specific for phosphorylation-dependent tau epitopes. The monoclonal antibodies PHF1, 12E8, and AT270 that are specific for Ser-396/Ser-404, Ser-262, and Thr-181, respectively, show similar patterns of phosphorylation in both gray and white matter (Figure 5 and data not shown). The antibody PHF6 that recognizes Thr-231 shows a relative increase in tau phosphorylation in gray matter; however, these differences are highly variable between cases (data not shown). In contrast, the antibody AT8 that recognizes Ser-202/Thr-205 demonstrates a relative increase in tau phosphorylation in white matter in most cases (Figure 5). The exceptions were two cases (patients 2 and 12) that biochemically demonstrate marked (3+) tau pathology in the frontal lobes. Similarly, by immunohistochemical analysis, AT8 preferentially recognizes the tau pathology in the white matter relative to other phosphorylation-dependent antibodies including PHF1 (Figure 6). The explanation for this observation is unclear, but the distinct pattern of phosphorylation may reflect differences in the subcellular localization (ie, cell bodies *versus* cell processes) of the tau pathology as AT8 preferentially recognizes the white matter pathology consisting primarily of threads derived from

Table 4. Insoluble Tau Pathology in CBD, Biochemistry

	Frontal lobe		Temporal lobe		Parietal lobe		Occipital lobe			Cerebellum	
Patient	Grey matter	White matter	Grey matter	White matter	Grey matter	White matter	Grey matter	White matter	Basal ganglia	Folia	White matter
1	1	1	3	3	2	2	1	1	3	1	1
2	3	3	3	2	2	2	0	0	3	1	2
3	3	3	2	1	3	3	2	2	3	0	1
4	2	2	2	2	3	2	2	2	3	1	2
5	2	2	2	3	2	3	0	0	3	0	0
6	1	2	1	1	3	3	0	0	2	0	0
7	3	3	3	3	3	3	3	3	3	0	2
8	1	1	1	1	1	1	0	0	2	0	2
9	1	1	NA	NA	2	2	2	2	3	2	2
10	2	1	NA	NA	2	2	1	2	3	NA	2
11	1	2	NA	NA	1	2	0	0	3	1	2
12	3	3	3	3	3	3	1	1	3	0	2

0, absent; 1, mild density; 2, moderate density; 3, high density; NA, not available.

multiple cell lineages including neuronal axons and glial processes.[25,36]

To determine whether the observed differences in gray and white matter reflect distinct patterns of tau isoform expression, we performed Western blot analysis of soluble tau extracts from gray and white matter. Dephosphorylated soluble tau extracts showed similar levels of tau isoform expression in both gray and white matter (Figure 7). The relative proportions of the six tau isoforms was similar to that observed in both AD (Figure 6) and normal (data not shown) brain tissue as previously reported with a ratio of 3R:4R-tau of ~1:1.[37]

Discussion

In this study, we assessed the distribution of tau pathology by both IHC and biochemistry in 12 patients with pathologically confirmed CBD. Similar to previous studies on CBD, there was heterogeneous and variable involvement of all cortices, although Armstrong and colleagues[38] reported similar pancortical tau pathology in CBD patients using a detailed morphometric analysis. In general, there was concordance between the biochemical and immunohistochemical data. We attribute the observed differences to the fact that the biochemical and IHC analysis were performed on contralateral hemispheres and thus reflects the well-defined asymmetrical nature of the disease. In contrast, the widespread distribution of tau pathology did not correlate with the clinical presentation of the patient. Several reports have demonstrated a correlation of neuron loss with clinical phenotype.[9,15,39] The absence of correlation in our study is probably due to several factors including: 1) the selection of specific brain regions to provide uniform analysis across all of the cases, rather than selecting the most affected region; 2) the analysis was performed at end-stage when the neurodegenerative process becomes more generalized; and 3) the tau pathology predates the cell loss that correlates with clinical phenotype.

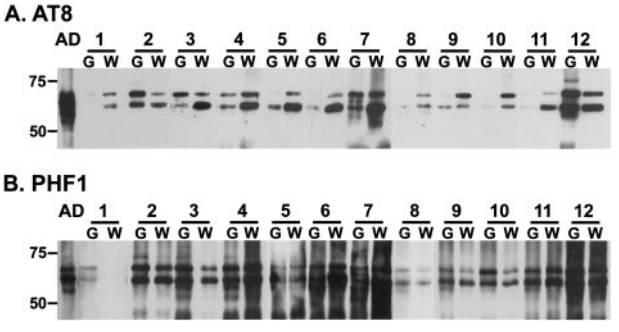

Figure 5. Western blot analysis of insoluble tau shows distinct phosphorylation profiles in white matter. Insoluble tau fractions from gray and white matter of the frontal lobe of CBD and AD patients were resolved by SDS-PAGE and immunoblotted with the epitope-specific, phosphorylation-dependent antibodies PHF1 and AT8 as indicated. PHF1 (**B**) detects similar patterns of phosphorylation in both gray and white matter. In contrast, AT8 (**A**), specific for phosphorylated Ser 202 and Thr 205, detects increased tau pathology in the white matter of the majority (10 of 12) of the cases. G, gray matter; W, white matter.

The composition of the insoluble tau aggregates in CBD is characterized by predominantly 4R-tau in both the neuronal and glial inclusions.[40–43] This is similar to that detected in PSP, but distinct from other disorders with extensive tau pathology such as AD and PiD in which aggregated tau is composed of either all six CNS tau isoforms (AD) or predominantly 3R-tau (PiD).[26,44] However, no previous studies of CBD included biochemical analysis of insoluble and soluble tau from multiple brain regions of many patients. In all 12 cases, we consistently detected insoluble tau composed of predominantly 4R-tau in both the gray and white matter of all brain regions with the exception of variable and typically mild pathology in the occipital lobe and cerebellum. Furthermore, similar to that observed in the frontal lobe, semiquantitative analysis indicated that there were typically equal, if not greater, amounts of pathology in the subcortical white matter relative to the gray matter of the same lobe.[40] The majority of the tau pathology in the white matter of CBD is in the form of threads, which implies that a significant majority of the insoluble tau pathology precipitates within processes rather than cell bodies in CBD. Consistent with this notion, Feany and Dickson[23] demonstrated that the thread pathology in CBD, particularly in white matter, was significantly greater than that observed in either PSP or PiD. Furthermore, in contrast to AD, co-localization studies suggest that a significant proportion of the thread

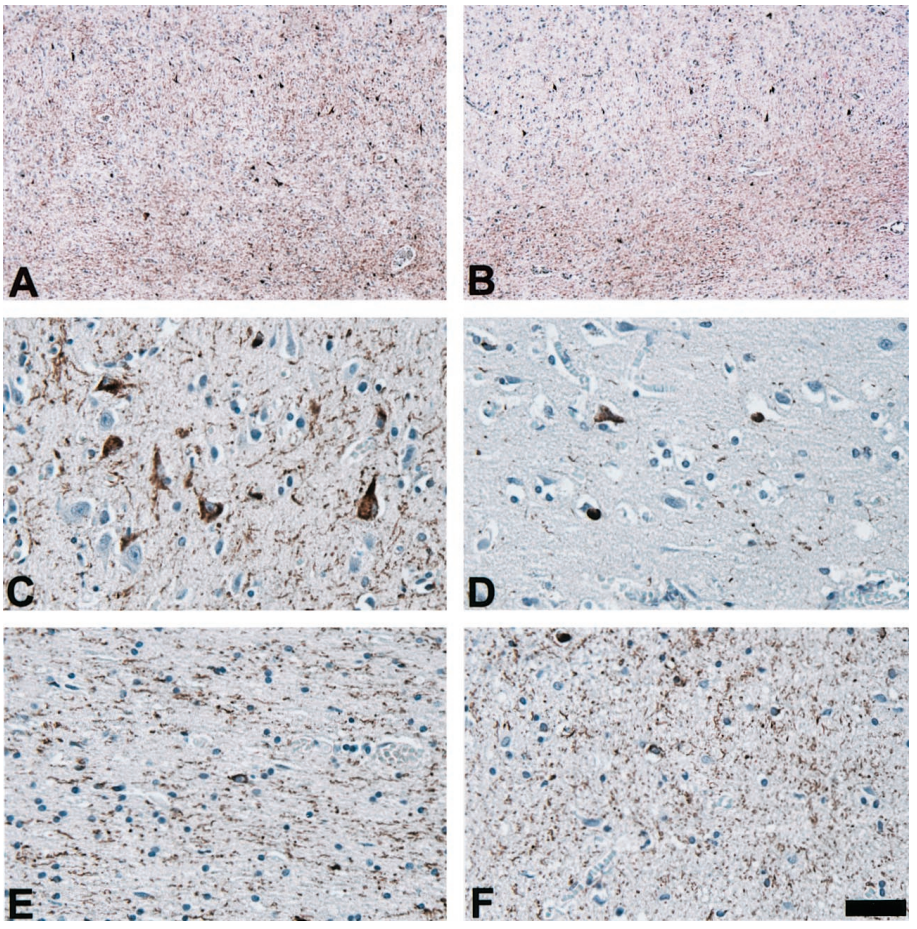

Figure 6. AT8 preferentially detects tau pathology in the white matter of CBD patients. Adjacent sections from the mid-frontal gyrus of patient 7 were immunostained with PHF1 (**A**, **C**, and **E**) or AT8 (**B**, **D**, and **F**). Although PHF1 detects abundant tau pathology in both the gray (**C**) and white (**E**) matter, AT8 preferentially detects the white matter pathology (**F**). **A** and **B:** Low-power photomicrographs of frontal lobe immunostained with PHF1 (**A**) and AT8 (**B**). **C–F:** High-power photomicrographs of cortex (**C** and **D**) and white matter (**E** and **F**) represented in **A** and **B** immunostained with PHF1 (**C** and **E**) and AT8 (**D** and **F**). Scale bars: 200 μm (**A** and **B**); 40 μm in (**C–F**).

pathology is within astrocytes and oligodendrocytes rather than neurons.[25,36]

In all tauopathies, including CBD, PSP, PiD, and AD, the insoluble tau is hyperphosphorylated and the vast majority of tau phosphoepitopes are identified in all of these disorders.[27,45] However, there appears to be a relative predominance of a subset of these phosphoepitopes in the white matter of CBD patients (Figure 5). Specifically, phosphorylated Ser-202/Thr-205 is relatively

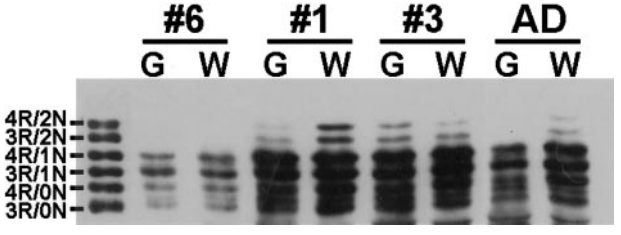

Figure 7. Western blot analysis of soluble tau shows similar levels of tau isoform expression in gray and white matter. Soluble tau fractions from gray and white matter of CBD and AD patients were dephosphorylated with *E. coli* alkaline phosphatase, resolved by SDS-PAGE, and immunoblotted with T14. The soluble extracts showed similar levels of tau isoform expression in both gray and white matter, similar to that observed in AD patients. Recombinant tau isoforms (rTau) are as indicated.

increased in white matter. Although the specific role of tau phosphorylation in its aggregation is not clear, we speculate that the abnormal phosphorylation of tau occurs by distinct mechanisms both within different cell types of the CNS and within distinct subcellular compartments (ie, cellular processes), thus accounting for the relative differences observed in the gray and white matter. Indeed, given recent evidence that *in vitro* phosphorylation of all six brain tau isoforms promotes filament formation, it is possible that in CBD the preferential accumulation of phosphorylated 4R-tau isoforms in glial tangles reflects a hitherto unexpected differential activity of specific kinases that preferentially target 4R-tau for phosphorylation.[46] However, the mechanisms for the observed differences in tau phosphorylation in gray and white matter remain to be elucidated.

The preferential aggregation of 4R-tau isoforms in CBD, as well as PSP, remains enigmatic. In FTDP-17, specific mutations that effect the splicing of exon 10 result in the aggregation of predominantly 4R-tau isoforms. However, this reflects the preferential expression of 4R-tau rather than a specific predilection of these isoforms to aggregate.[26–28] In both CBD and PSP, there

is no overt alteration in splicing of CNS tau relative to normal individuals or patients with tauopathies wherein all six tau isoforms (ie, AD) or preferentially 3R-tau (ie, PiD) aggregate. Interestingly, in both CBD and PSP, there is overrepresentation of the A0 allele, one component of the H1 tau haplotype.[47–49] The H1 haplotype consists of a series of polymorphisms in the tau gene, some of which are located within intron 10, that are in linkage disequilibrium.[47] The effect, if any, of the H1 haplotype or A0 allele is unknown, but it is possible that one or a group of polymorphisms specific to the H1 haplotype alter the biochemical properties or splicing of tau. However, to date, no association of the tau polymorphisms and splicing have been identified.

In conclusion, this present study supports the hypothesis that CBD has a distinct pathological phenotype characterized by the widespread aggregation of 4R-tau throughout both the gray and white matter of the CNS. However, although 4R-tau aggregates in a wide variety of cells, including astrocytes, oligodendrocytes, as well as neurons, the relative contribution of the glial pathology to the neurodegenerative process remains unknown.

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