Published in final edited form as:

Acta Neuropathol. 2013 May ; 125(5): 741–752. doi:10.1007/s00401-013-1087-8.

Corticobasal degeneration with olivopontocerebellar atrophy and TDP-43 pathology: an unusual clinicopathologic variant of CBD

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Abstract

CBD is a disorder affecting cognition and movement due to a progressive neurodegeneration associated with distinctive neuropathologic features, including abnormal phosphorylated tau protein in neurons and glia in cortex, basal ganglia, diencephalon and brainstem, as well as ballooned neurons and astrocytic plaques. We identified three cases of CBD with olivopontocerebellar atrophy (CBD-OPCA) that did not have α -synuclein-positive glial cytoplasmic inclusions of multiple system atrophy (MSA). Two patients had clinical features suggestive of progressive supranuclear palsy (PSP), and the third case had cerebellar ataxia thought to be due to idiopathic OPCA. Neuropathologic features of CBD-OPCA are compared to typical CBD, as well as MSA and PSP. CBD-OPCA and MSA had marked neuronal loss in pontine nuclei, inferior olivary nucleus, and Purkinje cell layer. Neuronal loss and grumose degeneration in the cerebellar dentate nucleus was comparable in CBD-OPCA and PSP. Image analysis of tau pathology showed greater infratentorial tau burden, especially in pontine base, in CBD-OPCA compared with typical CBD. Additionally, CBD-OPCA had TDP-43 immunoreactive neuronal and glial cytoplasmic inclusions and threads throughout the basal ganglia and in olivopontocerebellar system. CBD-OPCA met neuropathologic research diagnostic criteria for CBD and shared tau biochemical characteristics with typical CBD. These results suggest that CBD-OPCA is a distinct clinicopathologic variant of CBD with olivopontocerebellar TDP-43 pathology.

Keywords

Corticobasal degeneration; olivopontocerebellar atrophy; tauopathy; multiple system atrophy; progressive supranuclear palsy; TDP-43

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INTRODUCTION

Corticobasal degeneration (CBD) was first described in 1967 by Rebeiz, Kolodny, and Richardson as “corticodentatonigral degeneration with neuronal achromasia” [37]. CBD is a sporadic, mid-to-late life, adult-onset, neurodegenerative disease typically presenting with progressive levodopa-nonresponsive parkinsonism with focal cortical signs, such as apraxia, aphasia or frontal type dementia (reviewed in [32]). Pathologically, CBD is characterized by numerous neuropil threads in gray and white matter of affected cortices, basal ganglia, diencephalon, and brainstem; as well as characteristic astrocytic plaques [11] and ballooned neurons (BNs) [10]. Biochemically, abnormal tau in CBD is enriched in a specific alternatively spliced form of tau, namely, tau containing four ≈ 32 amino acid repeat motifs in the microtubule binding domain (4R tau), which is due to inclusion of exon 10 [5]. In addition to CBD, several other disorders are associated with abnormal accumulation of 4R tau, including progressive supranuclear palsy (PSP) [7] and argyrophilic grain disease (AGD) [39].

Olivopontocerebellar atrophy (OPCA) is a group of diseases pathologically characterized by neuronal degeneration of the inferior olivary nuclei, pontine nuclei, and cerebellum. The most common form of sporadic OPCA is multiple system atrophy (MSA). MSA is an adult-onset neurodegenerative disease clinically characterized by varying degrees of parkinsonism, cerebellar ataxia, and autonomic dysfunction and was first recognized as a clinicopathologic entity by Graham and Oppenheimer [17]. Neuropathologic features of MSA include degeneration in substantia nigra, putamen, inferior olivary nucleus, pontine nuclei, and cerebellum, but with sparing of the cerebellar dentate neurons (reviewed in [25]). Intracytoplasmic argyrophilic and α -synuclein-immunoreactive inclusions of oligodendrocytes, referred to as glial cytoplasmic inclusions (GCIs), are found in affected regions in MSA [4,36].

In this study, we describe three patients with OPCA and pathologic features of CBD (CBD-OPCA) with no GCIs, an unusual combination that has not been previously reported. CBD-OPCA demographic, clinical, and neuropathologic features are compared to typical CBD, PSP, and MSA. In addition, we examined the biochemical profile of tau in CBD-OPCA, CBD, and PSP.

MATERIALS AND METHODS

Case selection

The three CBD-OPCA cases were identified from a series of brains submitted for review or consultation to the neuropathology laboratory at Mayo Clinic in Jacksonville between 1998 and 2006. Out of 81 CBD cases observed during that time period, three had macroscopic and microscopic features of OPCA. These three cases are compared to a set of control cases, including CBD, MSA, PSP, and neurologically normal controls ($n = 11$ for each group).

Tissue sampling and pathologic assessment

Most brains submitted for neuropathologic evaluation include one half of the brain (usually left) divided in a mid-sagittal plane fixed in formaldehyde and the other half frozen for biochemical and genetic studies. Thioflavin S fluorescent microscopy was used to assess Alzheimer-type pathology [35], and hematoxylin and eosin-stained sections were used to evaluate neuronal loss and gliosis. For quantitative analyses, samples were taken from pons and medulla, as well as a transverse section of cerebellar hemisphere (including deep white matter and dentate nucleus) and a sagittal section of the cerebellar vermis. Three non-overlapping images were randomly captured from H&E stained sections of pontine nuclei, inferior olivary nucleus, cerebellar dentate nucleus, cerebellar Purkinje cell layer, and

vermis for each case (1.5 mm × 1.25 mm section for cerebellar Purkinje cell layer in cerebellar hemisphere and vermis; 0.6 mm × 0.5 mm section for inferior olivary nucleus, pontine nuclei and dentate nucleus). Mean value of the number of surviving neurons in each image was counted manually for each case.

Statistical analyses were performed with statistics software (GraphPad Prism 4.03, GraphPad Software, CA) and a probability value of $P < 0.05$ was regarded as significant for all analyses. The differences were examined with One Way Analysis of Variance on Ranks. When there was a significant difference, either Holm-Sidak or Dunn's method was performed for pair-wise comparisons.

Immunohistochemistry

Multiple cortical and subcortical areas, including brainstem and cerebellum were processed for immunohistochemistry for α -synuclein and tau. Sections of formalin-fixed and paraffin-embedded specimens were cut a 5- μ m thickness and mounted on glass slides, immunostained with a polyclonal rabbit anti- α -synuclein antibody (NACP) [19]; a phospho-tau monoclonal antibody (CP13 [23] from Peter Davies, Albert Einstein College of Medicine, NY); a phospho-TDP-43 monoclonal antibody (ps409/410, 1:5000, Cosmobio Co., Tokyo, Japan), or a p62/sequestosome (p62 lck ligand, 1:1,000, BD Bioscience, Franklin Lakes, NJ, USA) and then processed on an autostainer (DAKO Autostainer Universal Staining System, DAKO) using 3, 3'-diaminobenzidine as a chromogen. After immunostaining, the sections were counterstained briefly with hematoxylin. The α -synuclein immunohistochemical methods have previously been shown to have high sensitivity and specificity for Lewy body and MSA pathologies [5,13].

The tau burden was analyzed in CBD-OPCA ($n = 3$) and typical CBD ($n = 11$). Three non-overlapping images were randomly captured from tau-immunostained sections of middle frontal gyrus, pontine nuclei, inferior olivary nucleus, and cerebellar dentate nucleus (0.3 mm × 0.25 mm section). The captured images were processed with image analysis software (MetaMorph Offline version 7.0r4, Molecular Devices Corporation, CA). A ratio of the immunoreactive pixels to the total pixels of the whole field was calculated to yield an estimate of lesion burden (%) and the mean value was calculated for each case. In addition, the mean value of infratentorial tau burden (pontine base, inferior olivary nucleus and dentate nucleus) was calculated for each case. The differences were examined with an unpaired t-test.

Genetic analysis

All *MAPT* exons were amplified from genomic DNA for Cases 1 and 2. (No frozen tissue was available for Case 3.) PCR reactions of approximately 500 bp fragments were performed in 15 μ l reactions in 384 well plates. PCR products were purified using AMPure (Agencourt Biosciences) then sequenced in both directions using the BigDye Terminator cycle sequencing kit (Applied Biosystems). Sequencing reactions were purified using CleanSEQ (Agencourt Biosciences) and analyzed on an ABI 3730 Genetic Analyzer (Applied Biosystems). Base calling, sequence alignments and heterozygote detection was performed using Sequencher (Gene Codes Corporation). *C9ORF72* noncoding hexanucleotide repeats were examined as described in [8].

Tau biochemistry

A biochemical protein extraction followed a previously reported protocol [3]. Frozen brain samples from frontal cortex, pons, dentate nucleus of the cerebellum, and midbrain were homogenized into 2 volumes of TS buffer (50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 10 mM PMSF, 1 mM EGTA) and centrifuged at 20,000 g for 20 min. The resultant pellet was

resuspended and homogenized with 2 volumes of H buffer (10 mM Tris-HCl, pH 7.4, 0.8 M NaCl, 10 mM PMSF, 1 mM EGTA, 10% sucrose), and centrifuged at 100,000 *g* for 20 min. Resultant pellet (P1) was homogenized with 1% sarkosyl containing TS buffer and centrifuged at 100,000 *g* to obtain sarkosyl soluble supernatant (S3) and insoluble pellet (P3). The sarkosyl-insoluble P3 fractions were extracted with 2% sodium dodecyl sulfate (SDS) containing TS buffer. Each sample was mixed with SDS and β -mercaptoethanol containing sample buffer, separated on 12.5% Tris-glycine gels, transferred to nitrocellulose membranes (Millipore Co., USA), and immunoblotted. The primary antibody used against phosphorylated tau was PHF-1 [18] (1:1000, from Peter Davies, Albert Einstein College of Medicine, NY), which recognizes the C-terminal region of phosphorylated tau (p-Ser396/p-Ser404), and anti-mouse IgG secondary antibody (1:5000). MCID v6.0 image software (InterFocus Imaging, Cambridge, England) was used for densitometric analysis. The intensities of 37 and 40 kDa bands were calculated and divided by that of 33 kDa band in each case of CBD-OPCA, CBD, and PSP. The differences were examined by unpaired t-test.

RESULTS

Here we give a summary of neuropathologic features that were similar for all three cases, as well as clinical vignettes of the three patients with CBD-OPCA. Demographic information and clinical characteristics are summarized in Table 1. Tau pathology distribution and degree of severity for all three cases is provided in Supplementary Tables. DNA samples were available for Cases 1 and 2, and they were both negative for *MAPT* mutations in all coding exons. Fluorescent fragment-length analysis between noncoding *C9ORF72* exons 1a and 1b showed that both Cases 1 and 2 were heterozygous, and therefore, both alleles had hexanucleotide repeats of a nonpathogenic length. Only fixed tissue was available for study for Case 3. All three cases were of European descent. Immunohistochemistry for p62 was performed on cerebellum, medulla, and pons section and all three CBD-OPCA were negative for neuronal intranuclear inclusions.

Clinical features

Case 1—This 67-year-old woman had balance problems beginning at age 61, and she experienced multiple falls. Five years prior to her movement disorder, she suffered from depression following the death of her husband, and she became apathetic and withdrawn. One year into her illness, she exhibited a broad-based ataxic gait with an inability to tandem walk, abnormal Romberg, and a reduced stride length. Bradykinesia, rest tremor, and terminal fine tremor on finger-to-nose testing, and micrographia were observed. She was treated with carbidopa and levodopa with no apparent improvement. She then developed eye movement abnormalities including abnormal saccades, poor vision, reduced blink, and eyelid apraxia. Three years into her illness, she had vertical supranuclear gaze palsy, bilateral rigidity of the upper extremities, and cogwheel rigidity of the left arm. There was no apraxia, myoclonus or aphasia noted. Four years into her illness, neuropsychiatric evaluation showed significant cognitive impairment, especially in the domain of expressive language, and she had reduced concentration and a flat affect. In the final stage of her illness, she had dysphagia, hypophonia, increased rigidity, and urinary incontinence. She had episodes of confusion and agitation and did not spontaneously interact. She was diagnosed as clinically probable PSP.

Case 2—This 75-year-old man presented with balance problems at age 67. This progressively worsened, and he experienced several falls in the backward direction. His gait was wide-based, erect, and mildly ataxic. Five years into the disease, his speech became softened, and his extraocular motility showed vertical impairment. There was no resting or action tremor noted, but his reflexes were reduced throughout. He became bradykinetic, and

his ataxic gait progressively worsened. There was a paucity of spontaneous language output, mask-like facies, and eye movement abnormalities affecting both up and down gaze, with reduced blink rate. There was a positive family history for Parkinsonism in his brother. He was diagnosed with clinically probable PSP.

Case 3—This 74-year-old woman presented with an unsteady, ataxic gait and unexplained falls that began at approximately age 64. Her gait was wide-based, and she had difficulty walking in a straight line and on her toes and heels. She had normal jaw jerk, but had hyperreflexia of the upper extremities with bilateral Hoffmann signs. Reflexes were brisk at the knees and ankles without clonus. Detection of vibration at the toes and toe strength were slightly decreased. She had high-arched feet, with clawing of the toes. There was some difficulty on the finger-to-nose test with the upper limbs, right more than left, and there was no accentuation of this difficulty with the eyes being closed. There was mild dysarthria and mild weakness of the small muscles of the hands. Her speech became monotonic and did not modulate much. Decreased arm swing was noted with increased cogwheeling. Her handwriting deteriorated, and she had coordination difficulty with both of her hands. She had impaired alternative motion reactions of the right hand greater than left, but equally in both feet.

Neuroimaging—Brain MRI (1.5T GE Medical Systems Signa scanner, Milwaukee, WI) performed 5 years after the onset of symptoms showed a small brainstem, particularly from the pontomesencephalic junction to the pontomedullary junction (Fig. 4a). Sagittal and coronal T1-weighted MRI scan performed 7 years after onset of symptoms showed progressive focal cerebellar atrophy (Fig. 4a, b). T2-weighted MRI images demonstrated slightly increased signal within the transverse pontine fibers (Fig. 4c). Diffusion weighted imaging and fluid attenuated inversion recovery (FLAIR) images showing enlargement of the fourth ventricle and cerebellopontine cisterns (Fig. 4d). The olivopontocerebellar atrophy was bilateral. Her disease course was slowly progressive, and she died after 10-year disease duration. There was a possible history of multiple sclerosis in her mother and psychiatric illness in two siblings. This antemortem clinical diagnosis was idiopathic OPCA.

Neuropathologic features

Macroscopic—Cerebral atrophy was moderate in frontal lobe, and the corpus callosum was thin. The midbrain had loss of neuromelanin pigment in the substantia nigra, and the pontine base had marked atrophy (Fig. 1a). There was discoloration of the transverse fibers of the pontine base and middle cerebellar peduncle (Fig. 1b) with relative sparing of the trigeminal nerve on Luxol fast blue (Fig. 1b, arrow). The inferior cerebellar peduncle and cerebellar white matter were markedly atrophic with a dusky gray appearance (Fig. 1c) and had significant myelin staining pallor on Luxol fast blue (Fig. 1d, arrow).

CBD pathology—There were no GCIs with α -synuclein immunohistochemistry or Gallyas silver stains in all brain regions examined. On the other hand, the tau pathology in CBD-OPCA was consistent with CBD with the exception of an especially high tau pathology burden in the brainstem and cerebellum compared to that typically seen in CBD. Pretangles, neuropil threads, astrocytic plaques (Fig. 2a), and sparse coiled bodies were present in affected cortices and subjacent white matter. There were ballooned neurons in affected cortices (Fig. 2b). Sections from the frontal lobe showed diffuse gliosis and minimal microvacuolation. The substantia nigra had marked neuronal loss with extraneuronal melanin (Fig. 2c), axonal spheroids, and reactive astrocytosis. Some neurons in the substantia nigra and locus ceruleus had tau-positive inclusions typical of so-called “corticobasal bodies” [16] (Fig. 2c). The neuronal population of the subthalamic nucleus was relatively preserved, but there were numerous neuropil threads. Argrophilic grains

were present in medial temporal lobe structures, including the amygdala. Gliosis was observed in the striatum, globus pallidus, and thalamus.

TDP-43 immunohistochemistry—Sections from the medulla had the most striking TDP-43 pathology with many neuronal cytoplasmic inclusions (NCIs) in the inferior olivary nucleus, but not in the hypoglossal nuclei (Fig. 2d). Numerous TDP-43 immunoreactive glial cytoplasmic inclusions (GCIs) and threads were detected in the pontine base (especially pontocerebellar fibers), cerebellar white matter (Fig. 2e, f), subthalamic nucleus, globus pallidus, and substantia nigra. There were sparse TDP-43 positive NCIs in the pontine nuclei, but not in the pontine tegmentum, Purkinje cells, and cerebellar dentate nucleus. Detailed semi-quantitative TDP-43 immunohistochemistry scores for CBD-OPCA compared to typical CBD (n=4) are listed in Supplementary Tables S4–S6.

Olivopontocerebellar system—Sections from the medulla revealed marked neuronal loss in the inferior olivary nucleus and significant degeneration of olivocerebellar fibers (Fig. 3a). The pons was remarkable for severe neuronal loss in the pontine base and degeneration and gliosis of the transverse fibers (Fig. 3b) with preservation of the longitudinal fibers. The cerebellar white matter was atrophic and had marked demyelination (Fig. 3c). The cerebellum had patchy, but extensive neuronal loss in Purkinje and internal granular cell layers with many axonal torpedoes (Fig. 3c) and empty baskets. Tau-immunoreactive pretangles and numerous neuropil threads were detected in inferior olivary nucleus (Fig. 3d). Tau pathology was prominent in the pontocerebellar fibers (Fig. 3e), pontine base, and pontine nuclei had numerous pretangles. The cerebellar dentate nucleus had many pretangles and cerebellar white matter had numerous tau-immunoreactive threads and sparse coiled bodies (Fig. 3f). Cerebellar dentate nucleus had moderate neuronal loss and gliosis and pyknotic neurons with grumose degeneration.

Comparison of CBD-OPCA to MSA, typical CBD and PSP

Neuronal loss—Three CBD-OPCA cases were compared to CBD, MSA, PSP, and normal controls ($n = 11$ for each group) with respect to neuronal loss in key infratentorial nuclei involved in olivopontocerebellar degeneration. Clinical and demographic features of cases and controls used in quantitative analyses are summarized in Table 2. Neuronal loss was severe in the pontine nuclei and inferior olivary nucleus of CBD-OPCA and comparable to that seen in MSA (Fig. 5). PSP cases had pretangles and sparse globose type of neurofibrillary tangles (NFTs), but minimal neuronal loss in pontine nuclei and inferior olivary nucleus. Purkinje cell neuronal loss was most marked in MSA; less in CBD-OPCA and PSP; and minimal or absent in typical CBD. The cerebellar vermis, like the pontine nuclei and olivary nuclei, had a similar degree of neuronal loss in CBD-OPCA and MSA. Axonal torpedoes were more numerous in CBD-OPCA and MSA compared to typical CBD and PSP, although they were observed in all neurodegenerative disorders examined in this study. The cerebellar dentate nucleus had neuronal loss and gliosis in CBD-OPCA, but the dentate nucleus was spared in MSA and typical CBD. There was marked neuronal loss of dentate nucleus in PSP cases, which tended to be more severe than in CBD-OPCA. Grumose degeneration was frequent in both PSP and CBD-OPCA.

Tau immunohistochemistry image analysis

Image analysis of tau pathology was used to determine whether there were differences in severity of tau pathology in CBD-OPCA compared with typical CBD. Regional anatomical differences were assessed in middle frontal gyrus, pontine base, inferior olivary nucleus, and dentate nucleus of the cerebellum (Fig. 6). CBD-OPCA had more severe tau pathology in the pontine base ($P = 0.003$) compared with typical CBD. Although the results did not meet statistical significance, tau burden in the inferior olivary nucleus and dentate nucleus also

tended to be greater in CBD-OPCA compared with CBD. Since CBD-OPCA is rare and our cohort is small, we tested the mean tau burden of infratentorial structures by summing the burden in pontine base, inferior olivary nucleus and dentate nucleus; this analysis showed significantly greater tau burden in CBD-OPCA than in typical CBD ($P = 0.003$). CBD-OPCA also had significantly greater tau burden when including only the inferior olivary and dentate nuclei ($P < 0.05$, data not shown). In frontal cortex, the density of astrocytic plaques, coiled bodies, pretangles, and neuropil threads was similar in CBD-OPCA and typical CBD.

Tau biochemistry—We analyzed the biochemical profile of tau to determine whether tau protein accumulating in CBD-OPCA was similar to typical CBD. Immunoblot analyses of sarkosyl-soluble fractions for CBD-OPCA, CBD, and PSP was performed. CBD-OPCA, CBD and PSP all had prominent tau immunoreactive bands at approximately 64 and 68 kDa (Fig. 7a, asterisks). Both CBD-OPCA and typical CBD had prominent bands at 37 and 40 kDa (Fig. 7a, double arrows), whereas PSP had a single prominent band at approximately 33 kDa (Fig. 7a, single arrow). Quantitative analysis of PHF-1 immunoreactive tau bands showed significant differences in the ratio of 37 and 40 kDa tau to 33 kDa tau between both CBD-OPCA and typical CBD compared with PSP (Fig. 7b). Immunoblot analysis of sarkosyl-insoluble tau fragments with PHF-1 disclosed similar results (data not shown).

DISCUSSION

This study identified patients with pathologically confirmed CBD who had OPCA that clinically presented with either a symmetrical, atypical parkinsonism suggestive of PSP or a slowly progressive cerebellar syndrome suggestive of idiopathic OPCA. The typical asymmetrical rigidity and apraxia syndrome (*i.e.* corticobasal syndrome) of CBD was not present in any of the three cases. CBD-OPCA had marked pontine and cerebellar atrophy associated with severe myelin loss of the pontocerebellar fibers, middle cerebellar peduncle and cerebellar white matter. Yet, immunohistochemical analyses failed to show α -synuclein-positive GCIs that are pathognomonic of MSA [9], but instead revealed tau-immunoreactive astrocytic plaques, thread-like processes in gray and white matter, and ballooned neurons, all neuropathologic features of CBD. In addition, the biochemical profile of tau in CBD-OPCA was similar to that in CBD, supporting the idea that CBD-OPCA is actually a variant of CBD.

CBD-OPCA is one of several clinical variants of CBD

CBD can present with various clinical syndromes, with the particular clinical features dependent on the location of the greatest cortical involvement [2]. While many of the clinical presentations are asymmetric syndromes (*e.g.*, corticobasal syndrome or progressive nonfluent aphasia), symmetrical CBD is increasingly recognized [20] and such cases are often misdiagnosed as PSP, with an estimated frequency of 30% of pathologically confirmed CBD [31]. Disease duration and age of death were not significantly different between CBD-OPCA and previously reported CBD [27,32].

Previously, we identified distinct pathologic differences that accounted for CBD presenting clinically like PSP (a clinical syndrome also referred to as Richardson syndrome [42]), including more severe tau burden in white matter of the cerebellum and inferior olivary nucleus compared with CBD presenting with corticobasal syndrome. In the present study, we report that CBD-OPCA also has greater tau pathology in infratentorial structures, especially the pontine base (Fig. 6), indicating that greater hindbrain tau pathology plays a significant role in these atypical clinical presentations of CBD. The two CBD-OPCA cases diagnosed as PSP both exhibited an ataxic gait at some point during their illness, an atypical

feature for both CBS and Richardson syndrome, presumably reflecting the cerebellar involvement.

Cerebellar dentate pathology was highlighted in the first descriptions of CBD [37], but it is not a consistent finding in all cases, and it is not included in neuropathologic diagnostic criteria for CBD [10]. In contrast, dentate pathology, with grumose degeneration is very frequent in PSP [22,34]. The clinical significance of cerebellar dentate nucleus pathology in PSP is unknown. All three cases of CBD-OPCA in this small series had cerebellar dentate pathology comparable to that seen in PSP, which was much different from MSA, which had preservation of the neuronal population of the dentate nucleus. The neuronal density of the dentate nucleus of MSA was not only greater than PSP, CBD, and CBD-OPCA, but also control cases. Fukutani and coworkers reported that the increased neuronal density in the dentate nucleus in MSA is a counting artifact related to decreased dentate nucleus volume related to severe cerebellar atrophy [15], and this may also explain the results of the present study.

A caveat of our study is that we didn't use stereology to assess neuronal loss in the various key nuclei and that this could potentially cause bias in our evaluation. In order to ensure that randomly selected fields for neuronal counts were representative of the entire nucleus at the level where we sample the regions, we inspected the nuclei to ensure the neuronal loss was in fact diffuse and not focal. With the exception of the cerebellar dentate, macroscopic and light microscopic studies of CBD-OPCA have notable similarities to MSA with respect to neuronal loss in the olivopontocerebellar system. CBD-OPCA and MSA both had significant neuronal loss in pontine nuclei, inferior olivary nuclei, and cerebellar vermis. In MSA, it has been previously reported that the vermis is more severely affected than the cerebellar hemispheres [24], and this was also observed for both CBD-OPCA and MSA in the present study. Although CBD-OPCA and PSP had some degree of Purkinje cell loss, this was greatest in MSA compared to other groups.

CBD-OPCA is an uncommon variant of CBD

While there are a number of rare disorders that can produce familial and sporadic OPCA (the most common being MSA), none have been reported to be associated with tau pathology. On the other hand, Iwasaki and co-workers recently reported a Japanese patient with pathologically confirmed PSP who had severe olivopontocerebellar involvement and an antemortem clinical diagnosis of spinocerebellar degeneration [24]. MRI findings showed progressive brainstem and cerebellar atrophy similar to our Case 3. While there are several reports of sporadic tauopathies with accumulation of 4R tau [1,6,12,33], to our knowledge, this is the only report of OPCA associated with a 4R tauopathy. Review of the database of the Mayo Clinic brain bank, which includes referral PSP cases from CurePSP|Society for Progressive Supranuclear Palsy, with over 900 PSP cases, PSP with OPCA has not been detected. Therefore, OPCA appears to be more common in CBD than in PSP, although it is also quite rare in a series of pathologically confirmed CBD (3 of 81 cases (4%)).

The results of immunohistochemical and biochemical examinations lead to the conclusion that CBD-OPCA can be considered an uncommon, atypical variant of CBD, rather than a distinct 4R tauopathy. Tau immunohistochemistry showed astrocytic plaques in CBD-OPCA, which are useful in neuropathological diagnosis of CBD [9] and different from the tufted astrocytes (also known as "tufts of abnormal fibers") in PSP [30]. Different low molecular weight tau fragments originally reported in CBD and PSP by Arai and coworkers [3] were confirmed in the present study. Moreover, we show for the first time that CBD-OPCA resembles typical CBD and not PSP, favoring it being a variant of CBD rather than another type of 4R tauopathy. In our previous study of CBD presenting clinically with Richardson syndrome, we showed the usefulness of tau biochemical profile in differential

diagnosis. Despite clinical differences in CBD presenting with Richardson syndrome or with corticobasal syndrome, both had the same lower molecular weight tau species similar to those reported here [31].

All three cases of CBD-OPCA also had medial temporal 4R tau pathology, two of which were consistent with argyrophilic grain disease (AGD) [39,40]. It is not uncommon to find pathology of AGD in CBD, with almost 40% of CBD cases positive for AGD [38]. Interestingly, many patients with pathologically confirmed CBD presenting with Richardson syndrome also have increased limbic system tau pathology, with many cases meeting diagnostic criteria for AGD [31]. It is not clear if AGD *per se* is associated with a discrete clinical syndrome [26] and even less certain how presence of AGD modifies the clinical presentation of disorders in which it is a concomitant pathologic finding. Given the small number of cases in this study, it remains to be determined if medial temporal tau pathology consistent with AGD is always present in CBD-OPCA.

In addition to CBD and AGD pathology, all three cases of CBD-OPCA had abnormal TDP-43 immunoreactive neuronal and glial inclusions with a predilection for the olivopontocerebellar system. TDP-43 has been inconsistently detected in 4R tauopathies, being uncommon in PSP [43], but more frequent in AGD [14] and CBD [41]. The distribution of TDP-43 pathology in CBD-OPCA is unusual and, to our knowledge, has not been previously reported in CBD or any other neurodegenerative disorder. Given that it maps to the olivopontocerebellar system, which is severely affected by the disease process, suggests that it may be linked to neurodegeneration much as amygdala TDP-43 pathology is linked to neurodegeneration in Alzheimer's disease [21,28]. As in Alzheimer's disease, it is not clear if the TDP-43 is directly linked to the neurodegeneration or if it is secondary reaction to neurodegeneration. It is worth noting that we detected TDP-43 pathology in typical CBD cases, as well, but it was predominantly in diencephalon and basal ganglia. Thus, distribution of TDP-43 pathology even in typical CBD differs from that seen in frontotemporal lobar degenerations [29] and Alzheimer's disease [2]. Clearly, much remains to be learned about factors that contribute to selective vulnerability and concomitant pathologies in CBD and its clinicopathologic variants, including CBD-OPCA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Virginia Philips, Linda Rousseau, and Monica Castanedes-Casey for their expert technical assistance, Dr. Peter Davies at The Feinstein Institute for Medical Research, Manhasset, NY, for the gifts of CP13 and PHF-1 antibodies, and Dr. Rosa Rademakers for assisting with the genetic studies. Two of the CBD-OPCA cases and most of the PSP and CBD cases in this study were submitted to the CurePSP brain bank at Mayo Clinic in Jacksonville, FL; one was originally submitted to Department of Pathology and Laboratory Medicine diagnostic evaluation at Mayo Clinic in Rochester, MN. The studies would not have been possible without generous donations of family members and their contributions in this endeavor are greatly appreciated. This study was supported by NIH grants: P50-AG016574, P50-NS072187, CurePSP | Society for Progressive Supranuclear Palsy, The Robert E. Jacoby Professorship for Alzheimer's Research, and Mayo Foundation for Education and Research.

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Fig. 1. CBD-OPCA macroscopic features. The base of the brain shows prominent atrophy of the pons (*arrows* in a). A transverse section of the mid-pons shows marked atrophy of the pontine base, while the superior cerebellar peduncle is relatively preserved, and the trigeminal nerve (*arrow* in b) is prominent against the atrophic and has loss of myelin middle cerebellar peduncle (b). Cerebellum sections show marked white matter atrophy compared with normal control (c). Cerebellar white matter has loss of myelin (*arrow* in d). Luxol fast blue staining (b, d); case 1 (b, c); case 2 (d); case 3 (a)

Fig. 2.

Tau and TDP-43 immunohistochemistry in CBD-OPCA. Affected cortical regions show tau-immunoreactive astrocytic plaques (a) and ballooned neurons (b). The substantia nigra has neuronal loss and tau-positive “corticobasal bodies” (c). Abundant TDP-43-positive pathology was seen throughout the olivopontocerebellar system in CBD-OPCA, including neuronal cytoplasmic inclusions in the inferior olivary nucleus (d) and numerous threads and oligodendroglial inclusions in the pontine base (e) and cerebellar white matter (f).

Fig. 3. CBD-OPCA olivopontocerebellar system pathology. The inferior olivary nucleus has neuronal loss with degeneration of olivocerebellar fibers (a), marked degeneration of transverse fibers of the pontine base with preservation of longitudinal fibers (b), cerebellar white matter loss (c, *arrowhead*) and axonal torpedoes (c, inset *arrows*). The inferior olivary nucleus has many pretangles and threads (d). Pontine transverse fibers (e) and cerebellar white matter (f) with tau-immunoreactive threads. Luxol fast blue staining (a–c); H&E staining (c inset); tau immunohistochemistry (d–f); bar = 50 μ m

Fig 4. Brain magnetic resonance imaging (MRI) from Case 3. (a) T1-weighted mid-sagittal MRI section shows anterior cerebellar (*arrow*) and brainstem atrophy. (b) T1-weighted coronal section shows bilateral cerebellar atrophy. (c) Axial T2-weighted image and (d) fluid attenuated inversion recovery MRI showing enlargement of the fourth ventricle and cerebellopontine cisterns (*asterisks*).

Fig 5.

Neuronal loss in CBD-OPCA, CBD, MSA, PSP, and normal controls. Three non-overlapping images were randomly captured from H&E stained sections (1.5 mm × 1.25 mm section for cerebellar Purkinje cell layer in cerebellar hemisphere and vermis; 0.6 mm × 0.5 mm section for inferior olivary nucleus, pontine nuclei and dentate nucleus). Bars represent mean and error bars show the standard error of the mean. CBD-OPCA = corticobasal degeneration with olivopontocerebellar atrophy, MSA = multiple system atrophy, CBD = typical corticobasal degeneration, PSP = progressive supranuclear palsy. * $P < 0.001$ versus CBD-OPCA and MSA; § $P < 0.05$ (inferior olivary nucleus) and $P < 0.001$ (Purkinje and vermis) versus MSA; † $P < 0.05$ versus PSP

Fig. 6.

Image analysis tau burden in CBD-OPCA and CBD. The tau burden was quantified for CBD-OPCA ($n = 3$) and typical CBD ($n = 11$) on sections of middle frontal gyrus, pontine nuclei, inferior olivary nucleus, and cerebellar dentate nucleus ($0.3 \text{ mm} \times 0.25 \text{ mm}$ section). Boxes represent the ratio of tau immunoreactive pixels to total pixels as a % tau burden; median = black line; mean = white line; CBD-OPCA = corticobasal degeneration with olivopontocerebellar atrophy, CBD = typical corticobasal degeneration. * $P = 0.003$

Fig. 7.

Biochemical analysis of tau in CBD-OPCA, CBD, and PSP. Proteins were extracted from frontal cortex (F), pons (P), dentate nucleus of the cerebellum (D), and midbrain (M) of CBD-OPCA, CBD, and PSP. Sarkosyl-soluble fractions were separated by SDS-PAGE and immunoblotted (a). Tau was present at the characteristic doublet (~ 64- and 68-kDa, *asterisks*) for all cases, CBD-OPCA and CBD have a lower molecular weight doublet at ~37-kDa (*two arrows*, left) and PSP has a single band at ~33-kDa (*arrow*, right). (b) Densitometric ratio of 37- and 40-kDa to 33-kDa tau. Bar charts show mean and error bars show the standard error of mean. CBD-OPCA = corticobasal degeneration with olivopontocerebellar atrophy, CBD = typical corticobasal degeneration, PSP = progressive supranuclear palsy.

Table 1

Summary of demographics, clinical, and neuropathology of CBD-OPCA

	Case 1	Case 2	Case 3
Sex	female	male	female
Age at death, y	67	75	74
Disease duration, y	6	8	10
Family history	cousin: parkinsonism	brother: parkinsonism	mother: MS; 2 siblings: psychiatric
Initial clinical features	poor balance, unexplained falls; personality change	poor balance, unexplained falls; ataxic gait	gait ataxia, unexplained falls, hyperreflexia, high-arched feet
Later clinical features	ataxic gait, parkinsonism, vertical gaze palsy, cognitive impairment, dysphagia, hypophonia; L-DOPA nonresponsive	bradykinesia, vertical gaze palsy, hypophonia, mutism	ataxia, poor handwriting, pontocerebellar atrophy on MRI
Clinical diagnosis	probable PSP	probable PSP	idiopathic OPCA
Brain weight, g	1320	1220	1254
Braak NFT stage	0	II	I
Thal amyloid stage	1	3	0
Pathologic diagnoses	CBD-OPCA, AGD	CBD-OPCA	CBD-OPCA, AGD

Table 2

Summary of cases and controls used for quantitative neuronal counts

	M:F	Age at death, y (m $\pm$ SD)	Brain weight, g (m $\pm$ SD)	Braak NFT Stage (m $\pm$ SD)
CBD-OPCA	1:2	72.0 $\pm$ 4.4	1264 $\pm$ 51	1.0 $\pm$ 1.0
CBD	8:3	68.5 $\pm$ 7.8	1169 $\pm$ 111	1.2 $\pm$ 1.0
MSA	7:4	67.5 $\pm$ 7.5	1235 $\pm$ 141	1.4 $\pm$ 1.1
PSP	3:8	70.7 $\pm$ 7.85	1130 $\pm$ 95	1.7 $\pm$ 0.9
Normal controls	6:5	73.7 $\pm$ 12.8	1170 $\pm$ 159	1.6 $\pm$ 1.2

m $\pm$ SD = mean $\pm$ standard deviation