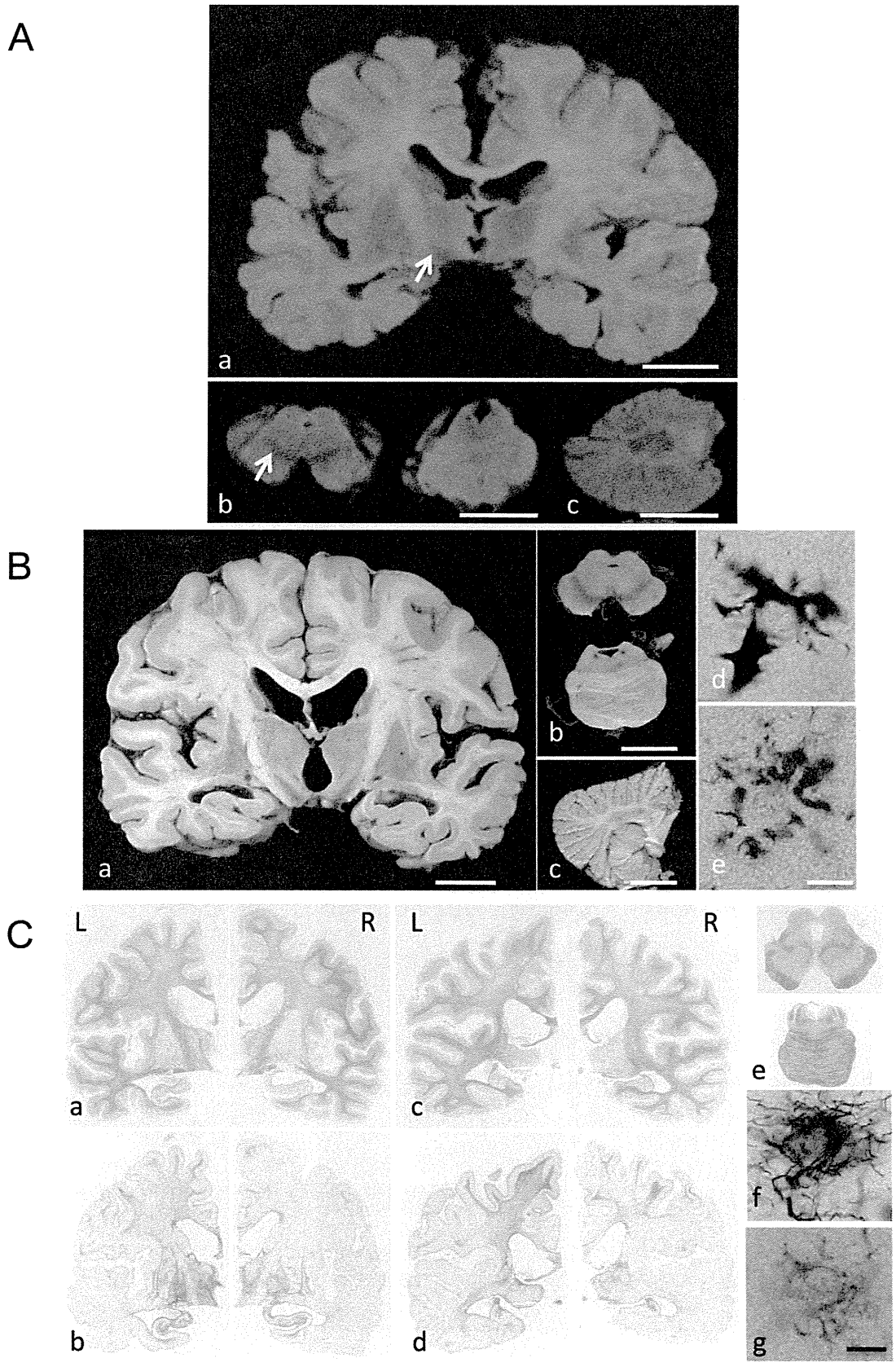


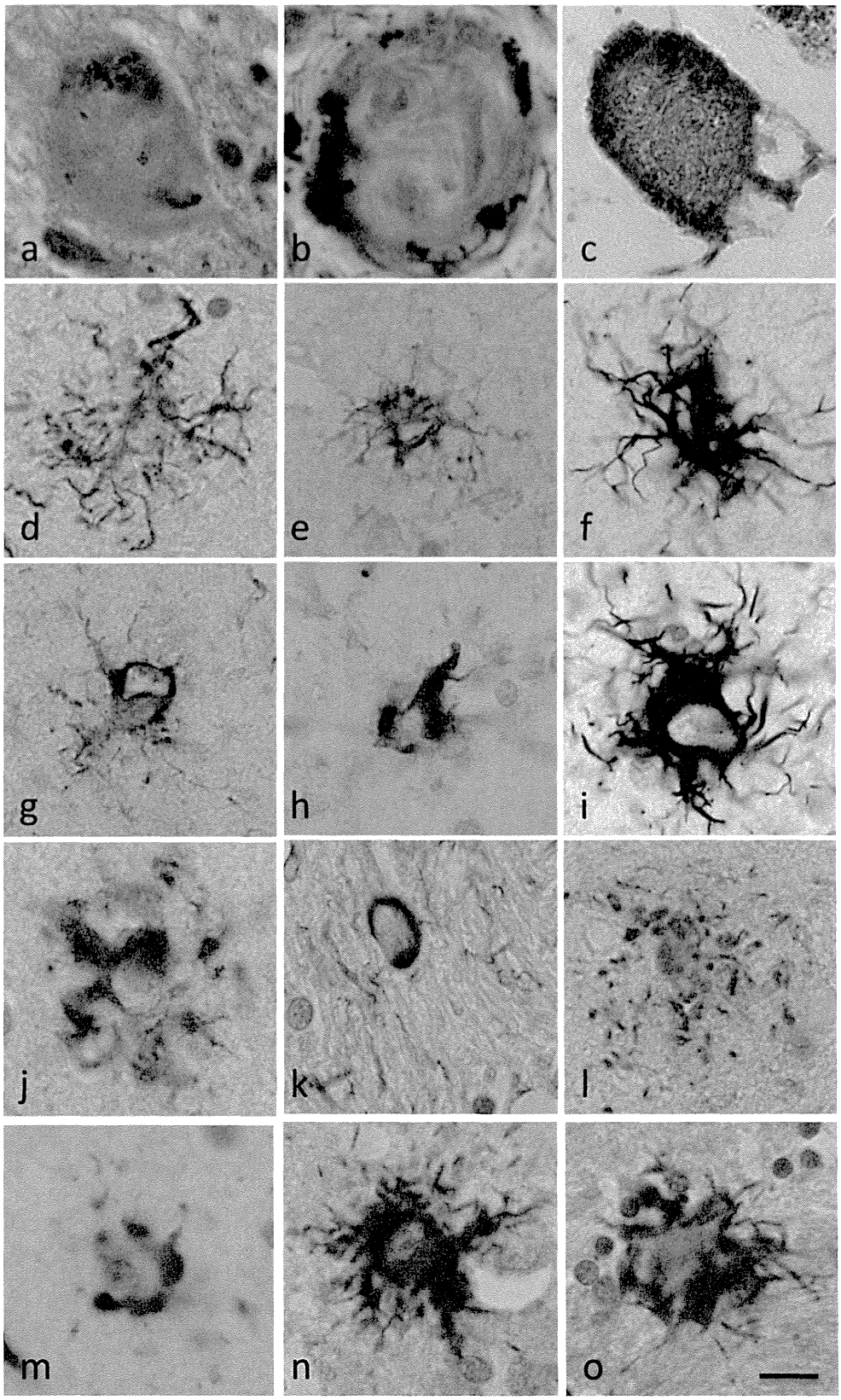


Fig. 3 Major microscopic findings in PSP. Microscopic findings include globose-type neurofibrillary tangles visualized with H&E stain (a), Bodian stain (b), and phosphorylated tau immunohistochemistry (c). Typical TAs have phosphorylated tau (d, g, j), 4 repeat tau (e, h) and Gallyas-positive (f, i) radiating proximal branches with variable cytoplasmic tau accumulation. Differential structures are oligodendroglial coiled bodies and threads (k) and tau accumulation in senile plaques (l), and globular glial inclusions in globular glial tauopathies (m). Double immunostaining for AT8 (brown) and GFAP (red) in TAs shows tau-positive radiating filamentous processes from the GFAP-positive cytoplasm (n) or more abundant tau accumulation in the proximal portion in the cytoplasm (o). a, H&E stain; b, Bodian stain; f, i, Gallyas silver stain; c, d, g, i–m, AT8 immunohistochemistry; e, h, RD4 immunohistochemistry; n, o, double immunostaining for AT8 (brown) and GFAP (red). Bar = 10 μm.

after either Gallyas-Braak (GB) silver stain or anti-tau immunohistochemistry; however, upon careful examination, some TAs revealed positive staining in the perikaryon after anti-tau immunohistochemistry (Fig. 3g). Electron microscopy demonstrated that astrocytes with abundant glial filaments formed loose bundles of straight 15 nm tubules in the perikaryon.[23] Fibers with a diameter of approximately 20–25 nm were also described.[21] Immunoelectron microscopy recognized TAs as central nucleus surrounded by radiating AT8-immunoreactive processes.[24] TAs are commonly found in the premotor, supplement, and motor cortex in the convex areas, the basal ganglia, thalamus, and brainstem tegmentum, and are recognized as a distinctive pathological marker of PSP.[25] With regard to disease specificity, it is strongly believed that the 4R tau-positive TAs are more common in PSP. It is probable that tufted astrocytes are the pathognomonic hallmark of PSP.[6,25,26]

Correlation between TAs and clinicopathological features

Based on the distribution of neuronal loss with gliosis and tau positive neuronal and glial inclusions, the pathological topography of 68 out of 70 PSP cases (except for 2 cases within one year's disease duration), were divided grossly into 3 representative subtypes: typical PSP type, pallido-nigro-luysian type (PNL-type), and CBD-like type (Fig. 1A,2). The typical PSP type (50 out of 68 cases, 73%) represents the prototypical pathological distribution, which involves the pallidum, subthalamic nucleus, substantia nigra, brainstem tegmentum, cerebellar dentate nucleus, thalamus and the cerebral cortices to a mild or moderate degree (Fig. 2A). SCD-like type (11 cases, 16%) involves severe degeneration of the dentate nucleus and atrophy of the pontocerebellum with tau accumulation (Fig. 1A). Because the SCD-like type normally develops into a typical PSP type, this type seems to be of similar etiology as the typical PSP type. The PNL-type (12 cases, 18%) demonstrates lesions that are relatively confined to the pallidum, subthalamic nucleus and substantia nigra (Fig. 1A,2B). CBD-like type (6 cases, 9%) reveals prominent asymmetrical cortical involvement that is associated with mild to moderate involvement of the brainstem and the cerebellar dentate nucleus (Fig. 1A,2C).

The pathology of the different subtypes wholly corresponds to the clinical phenotypes: pathologically typical PSP type corresponds to PSP-Richardson, pathological PNLA-types to PSP-PAGF or some PSP-P clinical phenotypes, and CBD-like type to PSP-corticobasal syndrome (CBS).[27] Therefore, the clinical phenotypes depend on the topographical distribution and the degree of degeneration. Furthermore transitional pathology also exists among those pathological subtypes in their distribution and severity.

In all subtypes, TAs are almost always invariably present. In the typical PSP type, TAs are usually prominent in the frontal cortices and in the striatum.[6] In the CBD-like type, more prominent TAs are seen in the affected cerebral cortices. In the PNLA type, typical TAs are less prominent, and atypical astrocytic tau inclusions are sometimes observed (Fig. 2B). These astrocytes in the striatum develop marked proximal dominant tau accumulation, but these atypical astrocytic tau inclusions are always accompanied by a few typical TAs (Fig. 3d–f). TAs in the cerebral cortices develop alongside NFT formations and other glial structures such as coiled bodies and threads, although TAs are already found early in PSP cases (within one year) and are even found in cases without formation of significant NFTs or other glial inclusions. Therefore, TAs do not necessarily accompany neuronal loss and reactive gliosis, but rather, they reflect intrinsic tau aggregation in astrocytes.[28]

Differential structures are tau-positive dystrophic neurites that surround senile plaques, thorn-shaped astrocytes, astrocytic plaques, coiled bodies, threads, and globular glial inclusions in globular glial tauopathies,[29] although these structures are generally well-differentiated based on each of their characteristic distributions and morphology, or by using immunohistochemistry to detect tau or Aβ (Fig. 3k–o).

CORTICOBASAL DEGENERATION (CBD)

CBD is a rare, progressive neurological disorder characterized by widespread hyperphosphorylated 4R tau pathology in neurons and glia in both cortical and subcortical areas.[4] The clinical presentation originally described by Rebeiz *et al.* involves asymmetric motor dysfunction including prominent involuntary movements.[2] Additional reports

have described similar patients who, in addition, demonstrated supranuclear gaze palsy and Parkinsonian features.[27,30-32] Although the combination of asymmetric motor disturbances with cortical sensory loss and apraxia without marked cognitive dysfunction has been thought to be specific for CBD, neuropathologically documented presentations as PSP and Parkinson's disease make antemortem diagnosis of CBD difficult.[33-35]

CLINICAL ASPECTS

In light of advances in the understanding of CBD, new guidelines have recently proposed 4 CBD phenotypes: CBS, which presents as an asymmetric movement disorder combined with lateralized higher cortical features, frontal behavioral-spatial syndrome (FBS), a nonfluent/agrammatic variant of primary progressive aphasia (naPPA), and progressive supranuclear palsy syndrome (PSPS).[36]

Neuropathology

Neuropathological diagnostic criteria

Due to the uncertainty and heterogeneity of the clinical criteria for the diagnosis of CBD, neuropathological examination is the gold standard used to make a definitive diagnosis. Clear pathological criteria have been proposed that were not adversely influenced by clinical information.[5] These criteria emphasize tau-immunoreactive lesions in neurons, glia, and cell processes in the neuropathologic diagnosis of CBD, although cortical atrophy, ballooned neurons, and degeneration of the substantia nigra have been emphasized in previous descriptions. The minimal pathologic features required for a diagnosis of CBD are cortical and striatal tau-positive neuronal and glial lesions, especially APs and thread-like lesions in both white matter and gray matter; neuronal loss in focal cortical regions and in the substantia nigra are also prerequisites for a diagnosis of CBD (Table 1).

Reevaluation of the neuropathology in CBD cases

Among the 30 pathologically confirmed CBD cases from our institute, 3 cases were associated with other neurological diseases: one with motor neuron disease with TDP-43 proteinopathy, one with DLB, and one demonstrated a clinical history of cerebral palsy. The 27 cases that were analyzed had a mean age at onset of 63 years (range 51–79 years), a mean disease duration of 6 years (range 3–13 years), and a mean age at death of 69 years (range 54–86 years). Although CBD is most often a sporadic disease, approximately 7% of affected individuals have a family history of neurological disorders, including PSP or dementia.

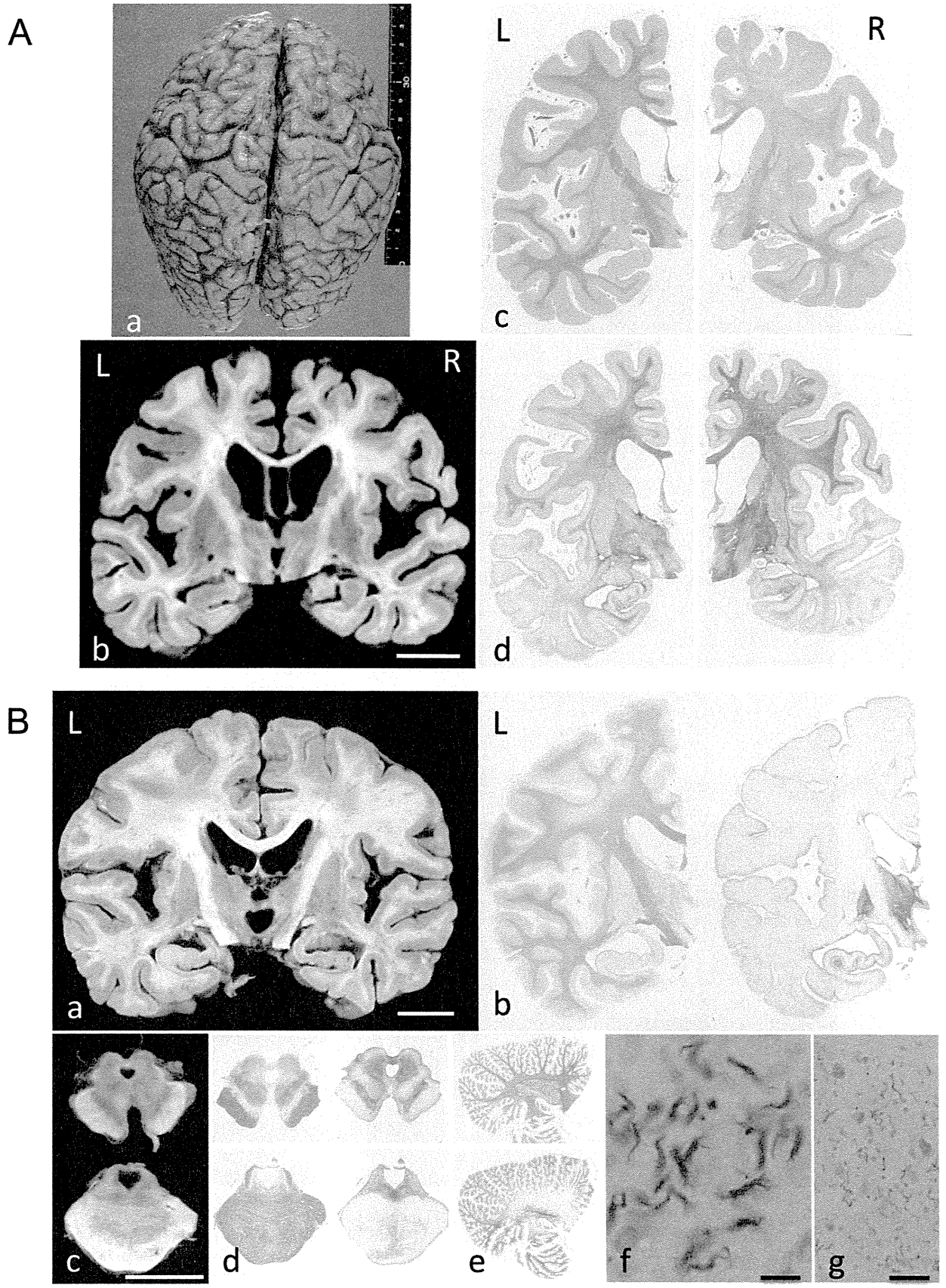
Macroscopic and microscopic findings

Typical macroscopic findings (Fig. 4A) include hemiatrophy or bilateral asymmetrical cortical atrophy, which predominates in the perirolandic area, posterior-frontal to the parietal area, anterior frontal, or in the perisylvian area. Coronal sections demonstrate thinning of cortical ribbons associated with rarefaction of subcortical white matter in the affected cortices. Medial temporal structures are relatively preserved, but the brainstem and cerebellum may show variable atrophy. The substantia nigra consistently shows severe depigmentation, with variable atrophy of the basal ganglia including the globus pallidus and the subthalamic nucleus.

The characteristic pathological hallmark of CBD is the swollen achromatic neuron, or ballooned cell (Table 1, Fig. 5a) that is present in affected cortical areas; however, ballooned neurons are nonspecific, and their quantity may vary according to the severity of the cortical involvement. Neuronal loss and gliosis are seen in the affected cortices, and typically, the degeneration of subcortical white matter is severe (Fig. 4Ac,d). Prominent cell loss and gliosis occur in the substantia nigra and are associated with varying degeneration of the globus pallidus and, to a lesser extent, of the subthalamic nucleus. The caudate and putamen show mild to moderate gliosis.

The pathological hallmarks of CBD are hyperphosphorylated 4R tau- positive neuronal and glial inclusions, pretangles, NFTs, threads, APs, and coiled bodies (Table 1, Fig. 5). Immunohistochemistry for the detection of hyperphosphorylated tau reveals these abnormal tau aggregates, but they are most conspicuous after GB silver staining.

Fig. 4 Macroscopic findings in typical CBD (A) and PSP-like CBD (B). (A) Macroscopic findings in typical CBD show right side-predominant bilateral cerebral atrophy (a). A coronal section demonstrates right side-predominant frontal atrophy, thinning of cortical ribbons and subcortical white matter, and atrophy of the globus pallidus and subthalamic nucleus associated with atrophy of the putamen and caudate (b). Medial temporal structures are relatively preserved. The same specimens as in panel (b) demonstrate right side-predominant atrophy of cortical and basal ganglia with reduced Klüver-Barrera staining of subcortical white matter including U-fibers (c), and this is shown by Holzer staining to be accompanied with fibrous gliosis (d). Bar = 2 cm. (B) Macroscopic findings in PSP-like CBD indicate severe atrophy of the pallidum and subthalamic nucleus with relatively mild degeneration of the cerebral cortices and subcortical white matter (a, b) and severe depigmentation of the substantia nigra accompanied by atrophy of the brainstem tegmentum (c, d) and dentate nucleus (e). Microscopically, astrocytic plaques are found in the basal ganglia and cerebral cortices (f). In the pontine nucleus, numerous pretangles, threads and neurites are found (g). b, d, e, Klüver-Barrera staining and Holzer stain. a, c, bar = 2 cm; f, bar = 10 μm; g, bar = 50 μm.



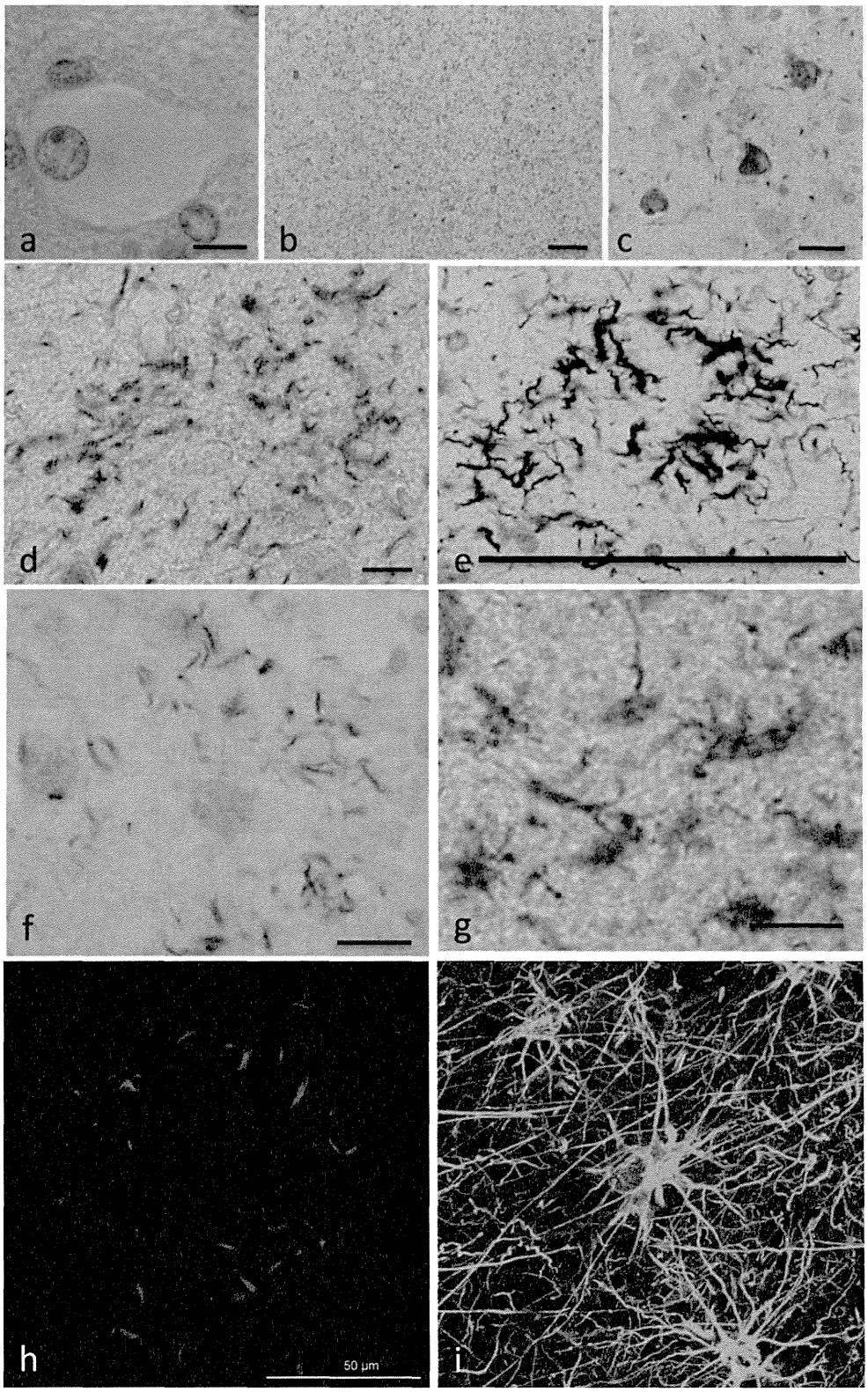


Fig. 5 Major microscopic findings in CBD. Microscopic findings in CBD include ballooned neurons (a), numerous tau-positive threads in cortical and subcortical structures (b), pretangles (c) and APs (d). An AP has Gallyas-positive (e) and 4R tau-positive (f) filamentous structures associated with collaterals (g) in a coronal arrangement. A double-immunofluorescence study of APs indicates a tau-positive coronal arrangement of filaments (h, red) located into the distal portion of GFAP-positive astrocytic processes (green), which are shown merged only within the distal portions (i). a, H&E; b, c, d, g, AT8; e, Gallyas silver stain; f, RD4 immunohistochemistry; h, i, double-immunofluorescence for tau (red) and GFAP (green). a, d, g, bar = 10 μm; b, bar = 50 μm; c, f, bar = 20 μm. e, bar = 100 μm.

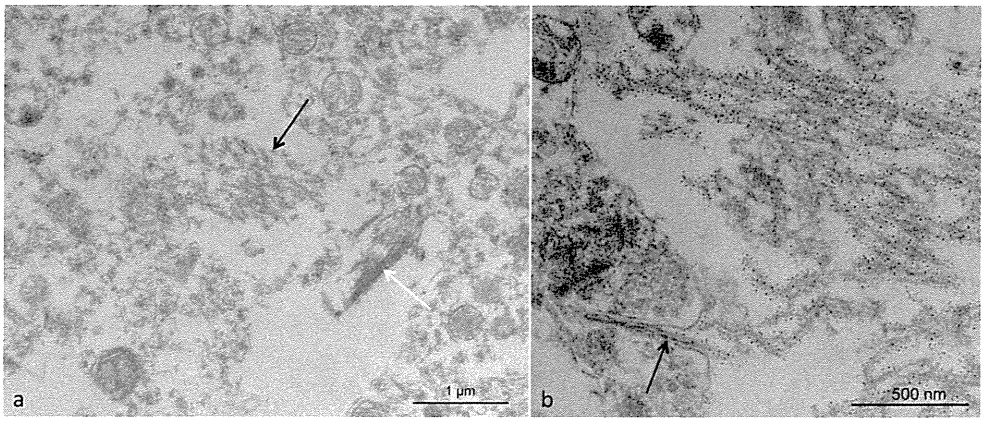


Fig. 6 Ultrastructural characterization of AP. Lower magnification of an AP, indicate Q dot-labeled AT8-positive short filaments (black arrow) loosely bundled near the synaptic structures and glial filaments (white arrow) (a). Higher magnification (b) indicates Q dot-labeled tau-positive short filamentous structures (black arrow) are inserted into a narrow gap near the synaptic structures. These short filamentous structures may represent the collateral structures of APs.

Although threads, pretangles and APs are highly specific for CBD, APs are almost exclusively seen in CBD and are considered a diagnostic hallmark.

Definition and differentials of astrocytic plaques

APs are not visualized in hematoxylin-eosin staining, but GB silver staining and tau immunohistochemistry reveal their distinctive morphology. APs exhibit a corona-like arrangement and are composed of fuzzy, short processes with tapered ends and fine collaterals, which were more evident on GB silver staining (Fig. 5d–g). No cytoplasmic staining was observed within the APs.[6]

APs were first identified by Feany and Dickson [37] using immunohistochemistry and laser confocal microscopy. They demonstrated that the nonamyloid cortical plaques of CBD are actually collections of abnormal tau in the distal processes of astrocytes, which were defined as APs. These glial cells express both vimentin and CD44, markers of astrocyte activation.

Differential structures are threads, TAs, other tau-positive astrocytic inclusions and senile plaques. Threads are fine, thin filamentous structures that are abundant in the cerebral cortices, subcortical white matter, basal ganglia, and brain stem; they are not corona-like in appearance and do not have collaterals or a bush-like appearance. Senile plaques are composed of amyloid β accompanied by tau-positive dystrophic neurites. TAs in PSP have been described previously.

Using double immunofluorescence staining, APs were found to be partially demonstrated colocalization with GFAP and tau in the distal portion of GFAP- positive asrtrocytic processes (Fig. 5h,i). Colocalization of GFAP and tau was not clearly observed in the cytoplasm or in the proximal processes of astrocytes. Immunoelectron microscopic examination was performed using the pre-embedding Q-dots immunolabeling, which was modified from the previously reported method.[38–40] It revealed that an AP contained a randomly arranged bundle of straight and twisted tubules in 15–20 nm diameter and the tau-positive filaments were densely immunolabeled with Q-dots (Fig. 6). Some of these filaments are located in a narrow hollow near synaptic structures (Fig. 6b). These findings suggested that the bush-like or collateral appearance of tau-positive APs may reflect the collaterals of distal astrocytic processes, which serve as a cover layer for the dendritic and perikaryal surfaces of certain neurons, and groups of, or individual synapses.[41] The location of filamentous tau accumulation of APs in the immediate vicinity of the synaptic structures suggest that APs may impair synaptic functions. APs were most abundant in the prefrontal and premotor areas in the cerebral cortex of brains from individuals diagnosed with CBD, but many APs were also observed in the caudate nucleus.[42] They are rarely seen in white matter with numerous tau-positive threads, despite severe gliosis. This also suggests that the morphology of the processes of protoplasmic astrocytes in the gray matter may be closely related to the corona-like structures of APs (Figs 7,8). The origin of tau-positive filaments in APs is still unknown. The density of APs is variable according to the degree and extent of the disease of the affected cortices. Even when the degeneration of the cerebral cortices is mild, a small number of typical APs are enough for a diagnosis of CBD.

Correlation between APs and clinicopathological features

Based on the distribution of neuronal loss with gliosis and tau positive neuronal and glial inclusions in the 27 CBD

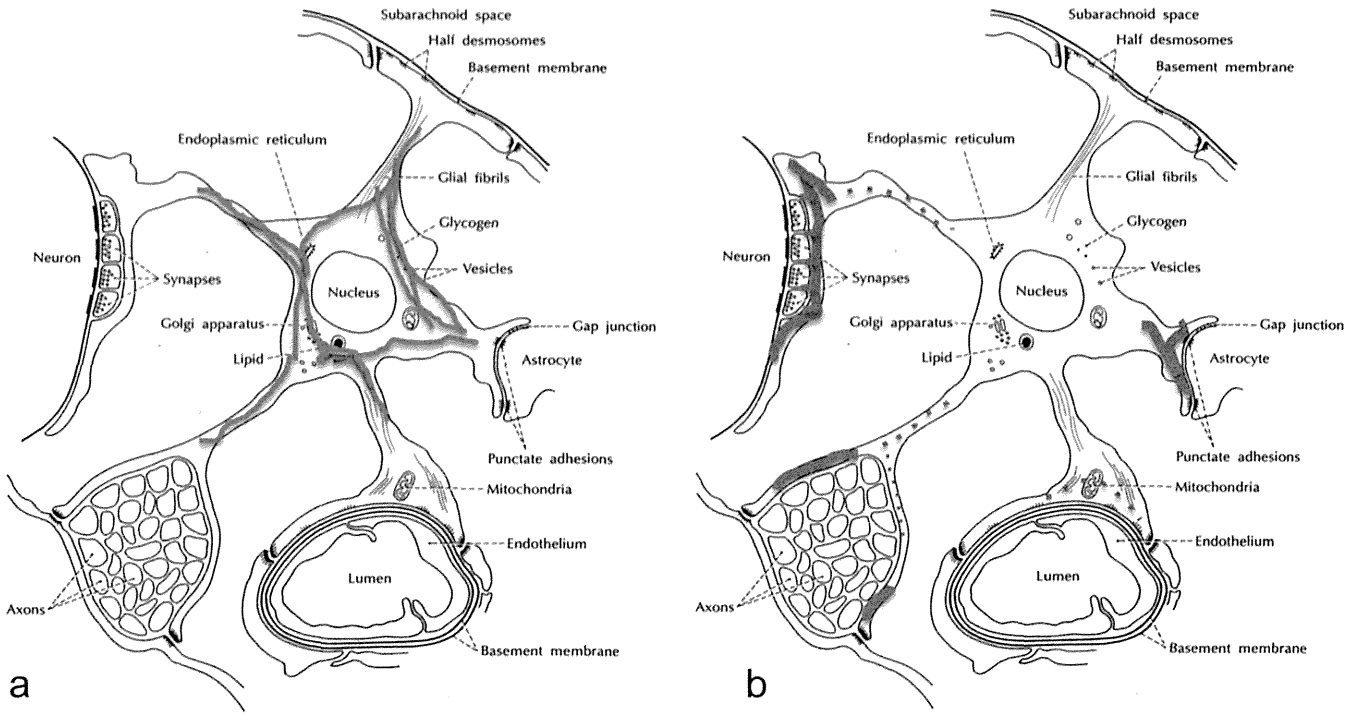


Fig. 7 Schematic illustration of tau accumulation in TAs of PSP and APs of CBD. In TAs (a), radially arranged filamentous tau aggregation (red lines) occurs in the cytoplasm and proximal processes of astrocytes. In APs (b), annularly distributed tau aggregation (green lines) is formed in the most distal portion of the astrocytic processes associated with collaterals. (Modified diagram reproduced with the permission of Igaku-Shoin from "A Guide to Neuropathology" by Hirano A, p205, Tokyo, 1981)[40]

cases, pathological topography was grossly divided into 3 representative subtypes: typical CBD type, basal ganglia predominant type, and PSP-like type (Fig. 1B). Typical CBD type (15 out of 27 cases, 56%) represents prototypical CBD and involves asymmetrical degeneration of the cerebral cortices and subcortical white matter, substantia nigra, pallidum, and the subthalamic nucleus, from a moderate to a severe extent (Fig. 1B). In the typical CBD type, the cortical distribution was predominantly in the posterior frontoparietal and perisylvian areas in 10 cases (37%), and in the anterior frontal area in 5 cases (19%). The PSP-like type (9 cases, 33%) revealed prominent involvement of the basal ganglia, brainstem and cerebellar dentate nucleus with mild to moderate involvement of the cerebral cortices without obvious laterality (Fig. 1B,4B). The basal ganglia-predominant type (3 cases, 11%) demonstrated relatively confined lesions to the pallidum, subthalamic nucleus, and the substantia nigra, with mild degeneration of the cerebral cortices, white matter, brainstem and cerebellum (Fig. 1B).[43]

The pathological subtypes wholly correspond to the clinical phenotypes: pathologically typical CBD type with posterior frontoparietal or perisylvian cortical lesions corresponds to CBD with clinical CBS; similarly, the pathological PSP-like type corresponds to CBD with clinical PSP syndrome. The pathologically typical CBD type with anterior frontal involvement corresponds to the clinical phenotype FBS, and the basal ganglia-predominant type demonstrates atypical parkinsonism or PSPS. Therefore, clinical phenotypes depend on the topographical distribution and degree of degeneration of the cerebral cortices, basal ganglia, and brainstem. In fact, we analyzed cases that exhibited borderline pathology between those pathological subtypes.

APs are exclusively seen in all CBD subtypes, although the density is variable according to the cerebral regions that are affected. APs are usually prominent in the frontoparietal cortices and in the striatum, especially in the caudate.[43] In the typical CBD type, more prominent APs are recognized in the affected cerebral cortices and in the striatum. In the PSP-like type or the basal ganglia- predominant type, APs are located in the putamen and caudate and to a lesser extent in the cerebral cortices. APs typically associate with pretangles and threads.

UNDERLYING MECHANISM

Although PSP and CBD are independent 4R tauopathies, highly overlapping pathological distributions seem to influence clinical diagnostic practices. The microscopic tau pathology allows for the clear discrimination of distinctive tau aggregates in neurons and glia between the two

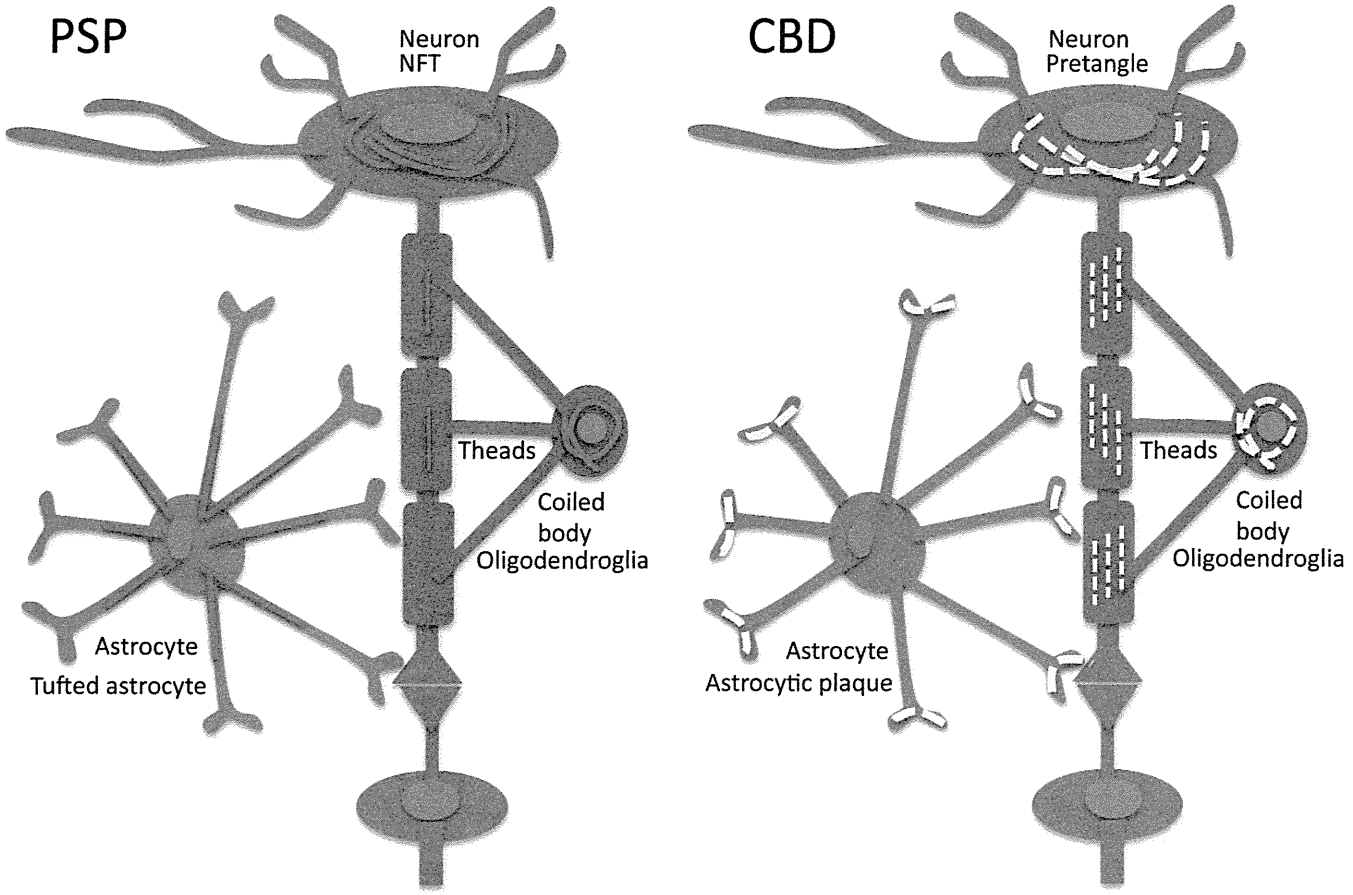


Fig. 8 Schematic illustration of tau accumulation in neurons and glia in PSP and CBD. In PSP (left panel), filamentous tau aggregation (red lines) is produced in the form of globose-type NFTs, TAs, and coiled bodies within the soma and proximal processes of neurons and glia. In CBD (right panel) less fibrillar tau aggregation (yellow lines) is produced and thus the majority of aggregates appear in the form of pretangles, APs, and threads within the cytoplasm of the soma and the distal processes of neurons and glia.

diseases. In this review, colocalization of TAs and APs was not found, although colocalization of TAs and APs as well as the coexistence of PSP and CBD in the same family were reported in some cases.[44–46] In the TAs of PSP, tau accumulates in the proximal portion of astrocytic processes and the perikaryon, in contrast to tau accumulation in the distal processes of astrocytes in the APs of CBD (Fig. 7). Tau accumulation of APs in the immediate vicinity of the synaptic structures suggests synaptic dysfunction by APs, which may provide the different pathophysiological mechanism from that of AD with predominant neuronal tau accumulation. Immunoelectron microscopic examination In Furthermore, in PSP, neuronal tau aggregates form a fibrillar, globose type of NFT, while in CBD, less filamentous tau accumulates in neurons as pretangles (Fig. 8).[47,48] On immunoblots of sarkosyl-insoluble brain extracts, a 33-kDa band predominated in the low molecular weight tau fragments in PSP, whereas two closely related bands of approximately 37 kDa predominated in CBD. These results suggest that despite the identical composition of the tau isoforms, distinct proteolytic processing of abnormal tau occurs in these two diseases.[49]

CONCLUSIONS

Astrocytic inclusions in PSP and CBD are briefly reviewed along with the pathological characteristics of tau aggregation and the pathological diversity of PSP and CBD. The different sites of tau aggregation in astrocytes and the different extent of fibril formation in neurons might help differentiate these two 4R tauopathies (Fig. 8).

Addendum

Dr. Nakano I. from Tokyo Metropolitan Neurological Hospital questioned the certainty of immunoelectron microscopic findings and the relationship between the APs and vessels. The double immunohistochemistry analysis of vimentin and AT8 in the frontal cortices of CBD patients indicated that the processes of astrocytes within APs rarely

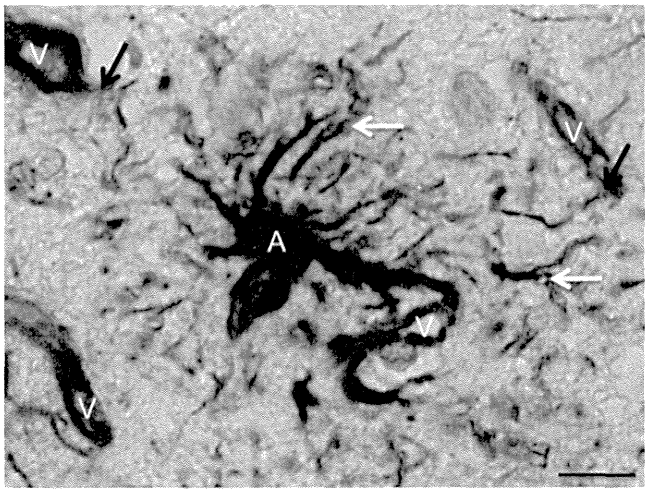


Fig. 9 Perivascular orientation of APs. Double immunohistochemistry for vimentin (violet) and AT8 (brown) of the frontal cortices in CBD indicates that the astrocytic processes radiate continuously to AT8-positive APs (white arrow) and rarely contact vessels (V), although some of the processes attached to the vessels showed AT8-positive staining (black arrow). V, vessel; A, astrocyte; bar = 10 μm.

contact vessels. Tau deposition into the perivascular foot processes was not remarkable, although some of the processes attached to the vessels demonstrated AT8-positive staining (Fig. 9).[50]

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The author declares no competing interests.

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REFERENCES

1. Steele J, Richardson C, Olszewski J. Progressive supranuclear palsy. *Arch Neurol* 1964; **10**: 333–359.
2. Rebeiz JJ, Kolodny EH, Richardson EP Jr. Corticodentatonigral degeneration with neuronal achromasia: a progressive disorder of the late adult life. *Trans Am Neurol Assoc* 1967; **92**: 23–26.
3. Hauw JJ, Daniel SE, Dickson D *et al.* Preliminary NINDS neuropathologic criteria for Steele-Richardson-Olszewski syndrome (progressive supranuclear palsy). *Neurology* 1994; **44**: 2015–2019.
4. Nukina N, Quan Y, Nakano I, Otomo E. Widespread tau abnormality in a case of cortico-basal degeneration. (In Japanese with English abstract. *Rinsho Shinkeigaku* 1992; **32**: 1093–1101.
5. Dickson DW, Bergeron C, Chin SS *et al.* Office of rare diseases neuropathologic criteria for corticobasal degeneration. *J Neuropathol Exp Neurol* 2002; **61**: 935–946.
6. Komori T, Arai N, Oda M *et al.* Astrocytic plaques and tufts of abnormal fibers do not coexist in corticobasal degeneration and progressive supranuclear palsy. *Acta Neuropathol* 1998; **96**: 401–408.
7. Williams DR, de Silva R, Paviour DC *et al.* Characteristics of two distinct clinical phenotypes in pathologically proven progressive supranuclear palsy: Richardson's syndrome and PSP-parkinsonism. *Brain* 2005; **128**: 1247–1258.
8. Williams DR, Holton JL, Strand C, Pittman A, de Silva R *et al.* Pathological tau burden and distribution distinguishes progressive supranuclear palsy-parkinsonism from Richardson's syndrome. *Brain* 2007; **130**: 1566–1576.
9. Williams DR, Holton JL, Strand K *et al.* Pure akinesia with gait freezing: a third clinical phenotype of progressive supranuclear palsy. *Mov Disord* 2007; **22**: 2235–2241.
10. Mochizuki A, Ueda Y, Komatsuzaki Y, Tsuchiya K, Arai T, Shoji S. Progressive supranuclear palsy presenting with primary progressive aphasia – Clinicopathological report of an autopsy case. *Acta Neuropathol* 2003; **105**: 610–614.
11. Josephs KA, Boeve BF, Duffy JR *et al.* Atypical progressive supranuclear palsy underlying progressive apraxia of speech and nonfluent aphasia. *Neurocase* 2005; **11**: 283–296.
12. Kanazawa M, Shimohata T, Toyoshima Y *et al.* Cerebellar involvement in progressive supranuclear palsy: a clinicopathological study. *Mov Disord* 2009; **24**: 1312–1318.
13. Iwasaki Y, Mori K, Ito M, Tatsumi S, Mimuro M, Yoshida M. An autopsied case of progressive supranuclear palsy presenting with cerebellar ataxia and severe cerebellar involvement. *Neuropathology* 2013; **33**: 561–567.
14. Litvan I, Hauw JJ, Bartko JJ *et al.* Validity and reliability of the preliminary NINDS neuropathologic criteria for progressive supranuclear palsy and related disorders. *J Neuropathol Exp Neurol* 1996; **55**: 97–105.