Results

TPD cases used in the present study

The demographic, pathologic, and clinical information of the TPD cases used in the present study is summarized in Tables 1 and 2. In general, both the clinical and neuropathological features are similar to those described in previous reports [1-3,7,26,27]. The average age at death is higher than that in AD. Moderate dementia was noted in 5 of the 7 cases but the other two were diagnosed as having mild cognitive impairment. Delusion was evident in 6 cases. Brain atrophy was mild, if present, and senile plaques were either absent or rare. Lacunar infarcts were seen in the globus pallidus in 2 cases. In all cases, heavy tau accumulation was seen in the limbic regions in the forms of NFT, diffuse cytoplasmic accumulations and neuropil threads. Tau accumulation was heavier in the subiculum and the CA1 region than in the entorhinal and transentorhinal cortices. Tau was also deposited in the amygdala, the septal nuclei and the basal nucleus of Meynert, and, less frequently, in the caudate nucleus and substantia nigra. A small amount of tau was found in the temporal neocortex but only in 3 cases. Such limbic-predominant distribution of tau pathology is consistent with previous reports [1,2,26,28]. A small number of argyrophilic grains were present in 2 cases.

Tau accumulation in the Acb in TPD

In addition to the previously reported tau distribution, we found a considerable number of tau positive neurons in the Acb in all TPD cases used in this study (Figures 1 and 2). Similarly to the hippocampus, numerous neuropil threads were associated with tau positive neurons (Figure 2A). The tau positive neurons and neuropil threads were labeled with all the anti-tau antibodies used in the present study (Figures 2A-D). They included conformational change-specific, phosphorylation-specific and phosphorylation-independent antibodies (Additional file 1: Table S1). The staining pattern varied, which partly depends on the affinity of the antibody and the localization of the antigen epitope recognized by each antibody. Preservation of the epitope in tissue sections is affected by aggregation, degradation and post-mortem processing such as fixation. The majority of tau positive neurons in the Acb showed pretangle-like, diffuse or granular accumulation of tau in the cytoplasm (Figures 2B). Flame-like NFT, the common form in the hippocampus in TPD, were also present but not frequent (Figure 2B, arrow). The vast majority of tau positive neurons were medium sized but, occasionally, large neurons were also stained positively for tau (Figure 2D, arrow). Tau positive neurons and threads were not distributed evenly in the Acb. Rather, areas with sparse- and dense-tau pathology were intermingled (Figure 2E).

Table 2 Demography and basic clinical and neuropathological features of TPD cases

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Age at death	89	102	90	85	89	78	86
Sex	F	F	F	F	F	M	F
Dementia	+	+	+	+	MCI	MCI	+
Psychiatric symptoms							
Delusion	+	+	+	+	+	+	-
Anxiety	+	-	-	-	-	-	-
Depression	-	-	-	+	-	-	-
Brain weight (g)	940	970	1170	1300	1230	1220	1130
Atrophy	mi(Fr)	mi(Fr/T)	-	-	-	-	-
Plaque stage (1)	0	0	A*	0	0	0	0
NFT stage (1)	III	III	III	III	IV	III	III
Argyrophilic grain stage (2)	0	0	0	0	II	II	0
Hippocampal sclerosis	-	-	+	-	-	-	-
Vascular lesions	+	-	-	+	-	-	-
α-synuclein (hip/T**)	+§	-	-	-	-	-	-
TDP-43 (hip/T**)	-	-	-	-	-	-	-
Acb tau score	3	2	2	3	3	2	3

F, Female; *M*, Male; *MCI*, Mild cognitive impairment; *mi*, Mild; *Fr*, Frontal; *T*, Temporal; *Acb*, The nucleus accumbens. *A small number of diffuse Aβ deposits were seen in the temporal cortex. The Acb tau score was determined according to the method described in the text. (1) The senile plaque and NFT staging were based on the description by Braak and Braak [6]. (2) The argyrophilic grain staging was based on the description by Saito et al. [29]. **Immunohistochemistry for α-synuclein and TDP-43 was performed in tissue sections of the hippocampus, parahippocampal gyrus and adjacent temporal neocortex. § α-Synuclein pathology in this case was mild, corresponding to stage 1 by the 3rd report of the DLB consortium [22].

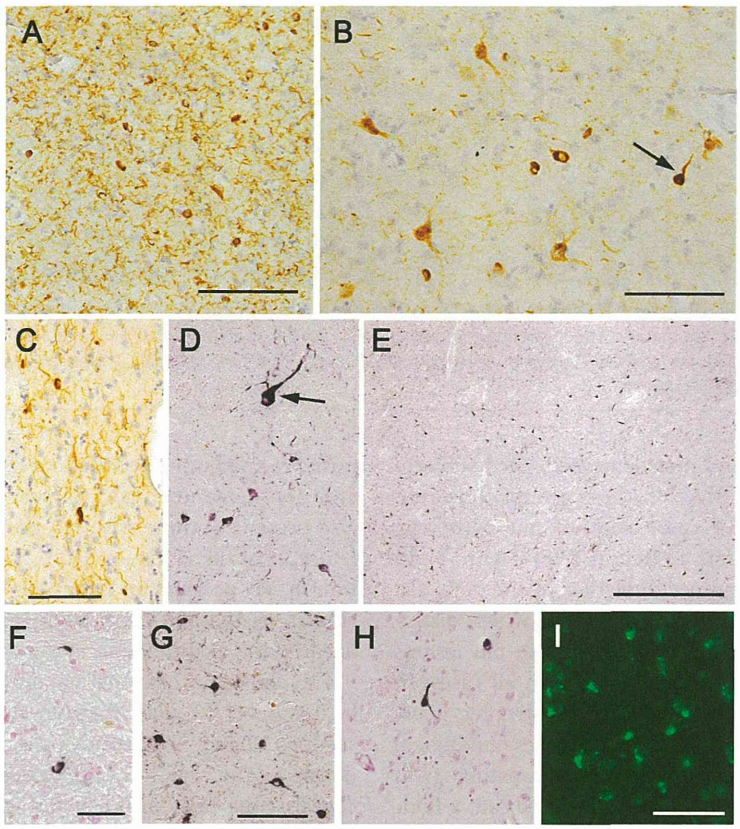

Figure 2 Tau accumulation in the Acb in TPD. A through **G** are immunohistochemistry with phosphorylation or conformational change specific tau antibodies. **A**, **B**, **C** and **I**: 4% paraformaldehyde-fixed, frozen-cut, 30 μm thick Sections. **D** through **H** are formalin-fixed, 10 μm thick, paraffin Sections. **A**: immunohistochemistry with AP422. Tau positive neurons are associated with many neuropil threads. Scale bars = 200 μm. **B**: immunohistochemistry with PHF-1. The majority of tau positive neurons show pretangle-like, diffuse or granular cytoplasmic labeling. Among them, apparent NFT are also seen but less frequently (arrow). Scale bar = 100 μm. **C**: immunohistochemistry with MC1, a conformational change specific antibody. Scale bar = 100 μm. **D** through **G** are immunohistochemistry with AT8. **D**: the vast majority of tau positive neurons are of medium-size but, occasionally, large neurons are also stained positively for tau (arrow). At the same magnification as **C. E**: tau positive neurons are not evenly distributed in the Acb. Scale bar = 500 μm. **F**: a glial coiled body. Scale bar = 25 μm. **G** and **H**: the nearby sections from the same case with AT8 immunohistochemistry **(G)** and Gallyas-Braak staining **(H)**. **I**: thioflavin S staining reveals granular cytoplasmic labeling of neurons. Scale bar = 100 μm.

Occasional glial coiled bodies were seen in the majoriy, if not all, of the cases (Figure 2F). Occurrence of glial coiled bodies in other brain regions in TPD has been reported previously [3]. Gallyas-Braak staining labeled only a small number of NFT in the Acb, while tau immunohistochemistry of nearby sections from the same patient revealed many positive cells (Figure 2G and 2H). Enhanced thioflavin-S staining labeled many neurons (Figure 2I).

The density of tau positive neurons and neuropil threads varied somewhat among the TPD cases. In TPD, no clear association was seen between the degree of Acb tau pathology and the Braak and Braak's NFT stage or the presence or absence of Aβ deposits, argyrophilic grains [29] and vascular lesions (Table 2). Despite the consistent tau accumulation in the Acb in TPD, we were not able to find severe neuronal loss or gliosis by HE staining.

Immunoelectron microscopy of the Acb in TPD with a tau antibody, AT8, revealed positive labeling of granular structures in the neurons (Figure 3A). Small and sparse bundles of short filamentous structures were occasionally seen to be stained positively for AT8 in the neuronal cytoplasm and neuropil (Figure 3B). Some of them showed morphology consistent with paired helical filaments (PHF). Thus, the ultrastructure of tau accumulation in the Acb was different from that in the hippocampal CA1 region, where dense and long bundles of PHF were frequent and intensely labeled for AT8 (Figure 3C).

Tau pathology in the Acb in AD and non-demented aged subjects

We then investigated the Acb in AD and non-demented, aged subjects. Tau positive neurons were found in some,

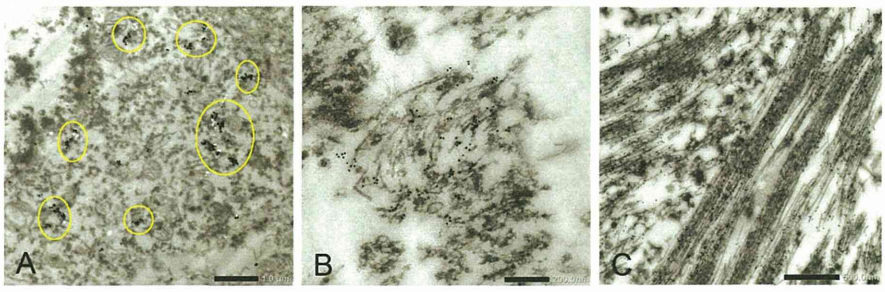

Figure 3 Immunoelectron microscopy of TPD brain with AT8 and immunogold labeling. A: in the Acb, the immunogold labeling in neurons was mostly localized to the granular structures (indicated by circles). Paired-helical filaments (PHF) were rare. Scale bar = 1.0 μm. **B**: sparse bundles of PHF were scattered in the neuronal cytoplasm. Scale bar = 200 nm. **C**: in the hippocampal CA1 region, prominent bundles of AT8 positive PHF were seen. Scale bar = 500 nm.

but not all, AD patients and non-demented, aged subjects (Additional file 2: Figure S1A). In these groups, however, only a limited number of cases showed tau pathology which was similarly abundant to that in TPD (Figure 4). In AD patients with heavy tau accumulation in the Acb, the caudate nucleus was also affected, a feature which distinguished AD from TPD. In TPD, the caudate tau lesions were either absent or, if present, very mild in all cases. In addition, senile plaques with tau positive dystrophic neurites were scattered in the Acb of such AD cases (Additional file 2: Figure S1B). In AD cases with mild tau pathology in the Acb, large neurons preferentially contained tau, a finding which was similar to the caudate nucleus in AD. In AD cases with heavy tau pathology in the Acb, such large neuron predominance became unclear and many tau positive, medium-sized neurons were seen. In both AD and non-demented, aged subjects, neuropil threads were also present in those with tau positive neurons in the Acb (Additional file 2: Figure S1C). The form of tau accumulation in AD patients and non-demented, aged subjects was similar to that in TPD, being predominantly pre-tangle like, diffuse accumulation in the cytoplasm.

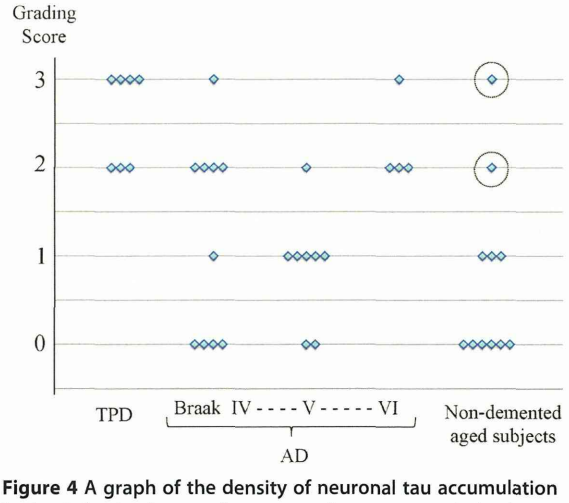

Figure 4 A graph of the density of neuronal tau accumulation in the Acb. The density of tau accumulation was graded as being 0 for absent, 1 for low, 2 for intermediate and 3 for high. Statistically significant differences were seen between TPD cases and non-demented, aged subjects ($P = 0.0031$) as well as AD cases in NFT stage IV ($P = 0.0192$) and V ($P = 0.0022$). The points encircled by a broken line in the non-demented, aged group indicate that the cases were over age 90.

The density of neuronal tau accumulation was graded to be 0 (absent) through 3(high) in AT8 immunostained tissue sections. Figure 4 illustrates the results in the Acb. Tau density in the Acb in the AD group was highly variable, except that the cases in Braak and Braak's NFT stage VI were either grade 2 or 3. Statistically significant differences were seen between the TPD cases and the non-demented, aged subjects ($P = 0.0031$) as well as the AD cases with NFT stage IV ($P = 0.0192$) and those with NFT stage V ($P = 0.0022$). Two non-demented, aged subjects with tau accumulation in the Acb were both over age 90. These 2 cases, similarly to TPD, lacked tau accumulation in the caudate nucleus and showed more NFT in the subiculum than in the entorhinal cortex. The results of semiquantitative analyses confirmed our observation that tau accumulation in the Acb was a remarkable finding in TPD. We performed similar analyses for a number of brain regions. The results are summarized in Table 3 as the averages of the graded scores for tau accumulation in each group. The concentration of tau pathology in the limbic structures, including the Acb and septal nuclei, in TPD contrasted with the broad distribution over the neocortex in AD.

Immunoblot analyses

The results of immunoblot analyses of samples from TPD and AD patients are shown in Figure 5. The tau band patterns in the sarkosyl insoluble fraction appeared to be essentially the same between TPD and AD, while the amount of insoluble tau was far smaller in the Acb than in the parahippocampal cortex in AD. It has to be noted that, because of the very high concentrations of insoluble tau in the parahippocampal cortex samples, the amounts of samples

Table 3 Summary of the semiquantitative grading of tau accumulation

	Braak stage	No. of cases	Acb	Caudate nucleus	Septal nucleus	CA1	Ent	Temp
TPD	III-IV	7	2.6	0.7	2.2	3	2.57	0.4
Non-demented	I-II	3	0	0	0	0.67	1	0
	III	8	1.13	0.25	0.63	1.38	2	1
AD	IV	10	1.2	0.6	1.4	2.67	3	1.4
	V	8	0.9	0.9	1.3	2.89	2.9	2.5
	VI	4	1.3	1.3	1.7	3	3	3

The numbers indicate averages of the scores in each group. The degree of tau pathology was qualitatively scored as 0: absent 1: low 2: intermediate 3: high.
TPD, Tangle predominant dementia; *AD*, Alzheimer's disease; *NFT*, Neurofibrillary tangles; *Acb*, nucl. Accumbens; *CA1*, Hippocampal CA1 region; *Ent*, Entorhinal cortex; *Tmep*, Temporal neocortex.

applied to the gels had to be reduced in AD cases. This resulted in the relatively weak signals for the Acb samples in AD cases. The dephosphorylated samples of the Acb and parahippocampal cortex showed the 3R + 4R isoform pattern in both TPD and AD.

The Acb tau pathology and the presence/absence of clinical history of delusion

Finally, we examined if the degree of the Acb tau pathology was different between the subjects groups with and without the history of delusion in the clinical records (Additional file 3: Figure S2). In the group of subjects with Braaks' NFT stages III and IV, which included NFT stage III non-demented aged subjects, all TPD cases and NFT stage IV AD cases, the Acb tau score was higher in those with clinical history of delusion than those without it ($p = 0.033$). Similarly, the less conspicuous but still significant difference was seen in the group of all NFT stage AD cases ($p = 0.049$).

Discussions

There is significant overlap in the distribution of NFT between TPD and AD. However, an early genetic study of TPD cases indicated a paucity of the apolipoprotein E ε4 allele, which currently is the most powerful risk factor for AD [30]. More recently, a report has been made on the significant associations of TPD with the *MAPT* H1 haplotype as well as with some polymorphisms within the region of *MAPT* encoding the 3′ UTR [31]. Thus, together with the striking paucity of Aβ deposition, it seems that TPD is a unique neuropathological entity that has to be studied separately from AD.

The clinical and neuropathological features of the TPD patients we used in the present study generally agreed with those described in preceding articles [3,32]. In

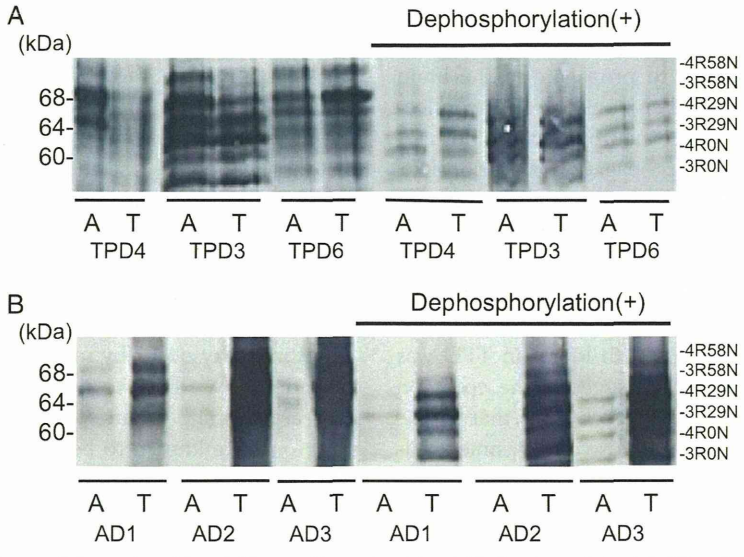

Figure 5 Immunoblot analyses of the sarkosyl insoluble tau. The sarkosyl insoluble fractions of the Acb (A) and the parahippocampal cortex (T) from TPD **(A)** and AD **(B)** were analyzed by immunoblot. A pan-tau antibody, HT7, was used. The Acb samples show the 3R + 4R isoform pattern similar to that in the parahippocampal cortices in both TPD and AD.

addition to the already well-known distribution of tau pathology, we found a considerable number of tau positive neurons and neuropil threads in the Acb. The Acb consists of the predominant medium-sized neurons and the occasional large neurons. Both cell types were affected by tau pathology in TPD. This contrasted AD with mild Acb tau pathology, in which large neurons were affected preferentially. Such a result is consistent with the previous reports which described that large neurons are more vulnerable in AD [33] and prone to tau accumulation [34]. In AD cases with the heavy Acb tau pathology, many medium-sized neurons were also tau positive. In the absence of TPD cases with the mild Acb tau pathology, it remains to be determined whether the difference is attributable to the distinct pathomechanisms between AD and TPD or to the variable vulnerability of different neuronal cell types.

The Acb may not be a region which is routinely sampled in a number of laboratories. Further, tau pathology in the Acb is not well stained by the Gallyas-Braak method. These facts together might explain the absence of previous reports on the occurrence of tau lesions in the Acb. The mechanism by which the Gallyas-Braak staining labels NFT remains to be determined. In TPD, insoluble tau consists of a mixture of the 3R and 4R isoforms in both the Acb and hippocampus, but NFT are intensely labeled by Gallyas-Braak staining in the latter. It may be noteworthy that, in the Acb, tau pathology occurs in a form of diffuse or granular cytoplasmic accumulations in the majority of tau positive neurons and that, ultrastructurally, PHF were rare. These contrasted with the hippocampal lesions where dense bundles of PHF were frequently seen. Obviously, factors that affect the reactivity of abnormal tau deposits to Gallyas-Braak staining need further clarification.

Tau pathology is considered to propagate in the brain from an affected region to another along the fiber connections, by spreading through the neuropil, or by both. A proposed mechanism for such propagation is a prion-like, seed-dependent conformational change and subsequent aggregation of the molecule, with a breakdown of the aggregate that generates the next seeds. In such a manner, the tau isoform pattern in the initial aggregates may be maintained in the later aggregate formations [35]. In the present study, we found that the Acb lesion in TPD was 3R + 4R tauopathy, a result which suggests the common origin of the tau pathology in the Acb with that in the hippocampus. The prevalence of diffuse cytoplasmic accumulations suggests that tau pathology in the Acb occurs later in the disease progression. In the hippocampus in TPD, many ghost tangles are seen, suggesting that the hippocampal lesions precede the Acb lesions.

Occurrence of NFT in the Acb in AD was reported previously [36,37]. In the present study, however, we have found that tau accumulation in TPD is more frequent and consistent than AD. The Acb receives direct and massive projections from the hippocampal CA1 and subiculum [14,38,39]. It has been repeatedly reported in TPD that the density of NFT is higher in the hippocampal CA1 and subiculum than in the entorhinal cortex [1,2,7,28]. Thus, the heavy tau pathology in the subiculum and CA1, through neural circuit-mediated propagation to the Acb, may result in the more pronounced tau accumulation in the Acb in TPD than in AD. Such an idea may be consistent with our finding that the difference in Acb tau pathology was statistically significant between TPD and AD in NFT stages IV and V but not in AD at stage VI. In AD with Acb lesions, tau accumulation was also found more frequently in the caudate nucleus than was the case in TPD. The caudate nucleus receives massive innervations from the cerebral cortex where, unlike TPD, tau pathology is severe in AD. On the other hand, the septal nuclei, like the Acb, receive direct projections from the subiculum and CA1 [14,38]. Again, we found, in the present study, heavier tau accumulation in these areas in TPD than in AD at NFT stages IV and V.

TPD is primarily an amnestic disease with relatively mild non-amnestic symptoms of dementia. In 6 of 7 TPD cases used in this study, however, delusion was a consistent clinical feature. This may be partly attributable to the fact that our brain tissue archive is principally based on the psychiatric hospital autopsies. However, occurrence of psychiatric symptoms has also been described in a number of previous reports on TPD. As an example, Jellinger et al. reported depression in 17.5% and paranoid ideas in 15% of TPD cases [3]. The Acb is part of the mesolimbic system in which the Acb receives dopaminergic input from the VTA. Recent evidence suggests that, in schizophrenia, functional abnormality in the Acb causes excessive release of dopamine from the VTA, which then results in the psychiatric symptoms [19,40-43]. While neuronal loss was not apparent in the Acb in TPD, it may be noteworthy that association of intraneuronal tau aggregation with clinical symptoms has been suggested in early stage AD lesions [44]. In AD, cases with more neocortical NFT were reported to be associated with more psychosis [45]. Thus, tau accumulation in the Acb could be related to the frequent delusion in TPD. Delusion and other psychotic symptoms may occur by multiple mechanisms in dementia patients. We have to note that 2 of the 7 TPD cases had argyrophilic grain pathology and that psychotic symptoms are known to be common in the patients with argyrophilic grain disease [46]. Whether the Acb tau accumulation is related to the psychiatric symptoms in TPD may be an issue for further investigation.

In the present study, we found that tau pathology occurred unevenly in the Acb in TPD (Figures 1 and 2E). The striatum is not uniform and has distinct neurochemical

compositions and connections that are referred to as matrix and striosomes. The similar but more complex compartmentation was reported in the human Acb [47]. We have performed additional immunohistochemistry for tyrosine hydroxylase (TH) and tau in serially-cut, free-floating sections in two TPD cases, in which the remnants of Acb blocks were available after the initial sectioning for the main body of this study. Comparison of the adjacent sections stained for TH and tau indicates that tau pathology preferentially occurs in areas where the fine, mesh-like TH staining is relatively light (Additional file 4: Figure S3). Such a result suggests the relationship between the uneven distribution of tau pathology and neurochemical heterogeneity in the Acb. However, because of the limited number of currently available samples and of the more complex neurochemical architecture in the Acb than the simple matrix-striosome structure in the caudate nucleus [47], future, extensive studies should be needed for further exploration.

Conclusions

We have found frequent tau accumulation in the Acb in patients with TPD. Both the medium-sized and large neurons are affected. While similar tau accumulation was seen in a small number of all AD patients, it was far more frequent and consistent in TPD than AD. The tau isoforms abnormally accumulated in the Acb were 3R and 4R, which suggests a common origin with the hippocampal tau pathology. The Acb receives direct and massive projections from the hippocampal CA1 and subiculum where tau pathology is extremely severe in TPD. Such a result may support the idea that abnormal tau aggregation propagates via neural circuits. Tau accumulation in TPD should be a subject of further investigations to approach the long-lasting issue of the simultaneous deposition of Aβ and tau in AD. In addition, the relationship between the tau pathology in the Acb and such psychiatric symptoms as delusion in TPD needs further exploration.

Additional files

Additional file 1: Table S1. The primary antibodies used in this study.

Additional file 2: Figure S1. Tau accumulation in the Acb in AD and non-demented, aged subjects. Immunohistochemistry with AT8. A: absence of tau positive neurons in an AD case in Braak and Braak's NFT stage IV. Scale bar = 100 μm in A-C. B: a diffuse cytoplasmic staining, neuropil threads and duystrophic neurite in a senile plaque in an AD case in NFT stage VI. C: a tau positive neuron and neuropil threads in a non-demented, aged subject.

Additional file 3: Figure S2. A graph of the density of neuronal tau accumulation in the Acb. The left plots: the group of Braaks' NFT stages III and IV, which includes non-demented aged subjects, TPD cases and AD cases with Braaks' NFT stage IV. Cases with delusion in the clinical history show higher tau score than those without delusion in the Acb. The right plots: the group of AD cases with Braaks' NFT stages IV through VI. Again, cases with delusion show higher tau score than those without delusion.

Additional file 4: Figure S3. The serial section immunohistochemistry for tau and tyrosine hydroxylase (TH). Forty micrometer thick, free floating sections were cut serially from two tangle predominant dementia (TPD) cases, in which the remnants of Acb blocks were available after the initial sectioning for the main body of the study. A set of every other section was stained for TH and the other set for tau with AT8. A and C: AT8 staining in a TPD case 1. B: TH staining of the section between A and C. In B, two types of areas are distinguished based on the modest difference in the density of fine, mesh-like TH staining. There is a propensity that tau pathology preferentially occurs in areas where the fine, mesh-like TH staining is relatively light (A, C). Scale bar = 2 mm in A (A, B and C are at the same magnification). D: higher power photomicrographs of the boxed areas in B and C. The left half is the staining with AT8 and the right half staining for TH. Scale bar = 400 micro-m (D).

Abbreviations

(TPD): Tangle-predominant dementia; (NFT): Neurofibrillary tangles; (AD): Alzheimer's disease; (3R): 3-repeat; (4R): 4-repeat; (Acb): Nucleus accumbens; (VTA): Ventral tegmental area; (HE): Hematoxylin and eosin; (PFA): Paraformaldehyde; (PHF): Paired helical filaments; (Aβ): Amyloid β protein; (TH): tyrosine hydroxylase.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

IK carried out the microscopic observation, immunoblot and statistical analyses. IK also drafted the initial manuscript. MHa conducted the sample preparation and immunoblot and carried them out with IK. TA participated in the design and coordination of the study. KI carried out the microscopic observation with IK. KO, KN and NA organized the brain archives including clinical information, selected appropriate cases, and performed neuropathological analyses of all cases used in this study. OK participated in the design of the study and performed statistical analyses with IK. SH conceived of the study and participated in the initial design. MHo contributed to the reagents, materials and analysis tools, and conducted free-floating immunohistochemistry. YH participated in the design of the study and helped to draft the manuscript. HA supervised the design and coordination of the study and worked up the manuscript. All authors read and approved the final manuscript.

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Symposium: Definition and Differentials – How to Distinguish Disease-Specific Changes on Microscopy

Significance and limitation of the pathological classification of TDP-43 proteinopathy

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Based on the cerebral tans-activation response DNA protein 43 (TDP-43) immunohistochemistry, frontotemporal lobar degeneration with TDP-43 pathology (FTLD-TDP) is classified into four subtypes: type A has numerous neuronal cytoplasmic inclusions (NCIs) and dystrophic neurites (DNs); type B has numerous NCIs with few DNs; type C is characterized by DNs which are often longer and thicker than DNs in type A, with few NCIs; and type D has numerous neuronal intranuclear inclusions and DNs with few NCIs. The relevance of this classification system is supported by clinical, biochemical and genetic correlations, although there is still significant heterogeneity, especially in cases with type A pathology. The subtypes of TDP-43 pathology should be determined in cases with other neurodegenerative disorders, including Alzheimer's disease and dementia with Lewy bodies, to evaluate the pathological significance of TDP-43 abnormality in them. The results of the biochemical analyses of the diseased brains and the cellular models suggest that different strains of TDP-43 with different conformations may determine the clinicopathological phenotypes of TDP-43 proteinopathy, like prion disease. Clarifying the mechanism of the conformational changes of TDP-43 leading to the formation of multiple abnormal strains may be important for differential diagnosis and developing disease-modifying therapy for TDP-43 proteinopathy.

Key words: fragment, phosphorylation, propagation, truncation, ubiquitin.

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HISTORY AND BACKGROUND

Trans-activation response (TAR) DNA-binding protein of M_r 43 kDa (TDP-43) is a major component of the tau-negative and ubiquitin-positive inclusions that characterize amyotrophic lateral sclerosis (ALS) and the most common pathological subtype of frontotemporal lobar degeneration (FTLD-U), which is now referred to as FTLD-TDP.[1–7] Several genes, including the *granulin* (*GRN*),[8,9] *valosin-containing protein* (*VCP*),[10] *TARDBP*[11–15] and *C9ORF72*,[16,17] have been reported to be associated with familial forms of FTLD-TDP and ALS. These disorders are now collectively referred to as TDP-43 proteinopathy.[1–4]

TDP-43 was first isolated as a transcriptional inactivator binding to the TAR DNA element of the HIV-1 virus.[18] It belongs to the group of 2 RNA-binding domain (RBD)-Gly RNA-binding proteins, which include the heterogeneous nuclear ribonucleoprotein (hnRNP) family and factors involved in RNA splicing and transport.[19]

It is known to be involved in multiple cellular processes, including gene transcription, alternative splicing and stabilization of mRNA, microRNA biogenesis, apoptosis, and cell division.[20–28]

Ubiquitin- and TDP-43-positive pathological inclusions found in FTLD-TDP include neuronal cytoplasmic inclusions (NCIs), dystrophic neurites (DNs), neuronal intranuclear inclusions (NIIs), and glial cytoplasmic inclusions (GCIs).[1,2,29–31] In ALS, motoneuronal skein-like inclusions (SLIs) immunopositive for ubiquitin had been regarded as one of the major pathological hallmarks.[32–34] Recent detailed immunohistochemical studies have clarified the wide distribution of neuronal and glial TDP-43 pathology in multiple areas of the CNS, including nigrostriatal system, the neocortical and allocortical areas, and the cerebellum.[35,36] These findings suggest that ALS does not selectively affect only the motor system, but rather is a

multisystem neurodegenerative TDP-43 proteinopathy affecting both neurons and glial cells.[35,36]

Biochemical analyses of the detergent-insoluble fraction extracted from brains of patients afflicted with FTLD-TDP and ALS show that TDP-43 accumulated in these pathological structures is phosphorylated, proteolytically cleaved and ubiquitinated.[1,2]

In the present review, we will focus on significance and limitation of the pathological classification of TDP-43 proteinopathy, based on the immunohistochemical and biochemical examinations of diseased brains.

DEFINITION AND DIFFERENTIALS

Definition of TDP-43 positive structures

NCIs (Fig. 1A,B)

The appearance of ubiquitin-positive tau-negative NCIs in the hippocampal region was first recognized in patients with ALS,[37] and was subsequently found in those with FTLD with motor neuron disease (FTLD-MND)[38,39] and in FTLD without MND.[40–42] TDP-43 positive NCIs are frequently found in the frontotemporal neocortex and the dentate granule cells of the hippocampus. Characteristically, by immunohistochemistry using phosphorylation-independent anti-TDP-43 antibodies, normal nuclear staining of TDP-43 is lost in neurons with NCIs.[1,3]

NIIs (Fig. 1B)

Ubiquitin-positive NIIs in FTLD-U were first reported in familial cases.[43,44] They have a lentiform or "cat's eye" appearance and are present in small neurons in multiple neuroanatomical sites. They are observed more frequently in familial cases than in sporadic cases.[35]

DNs (Fig. 1C)

Two types of DNs, short DNs and elongated DNs, are recognized.[45,46] They are positive for both TDP-43 and ubiquitin. Both types of DNs are typically most numerous in layer II of the frontal and temporal cortices, although the

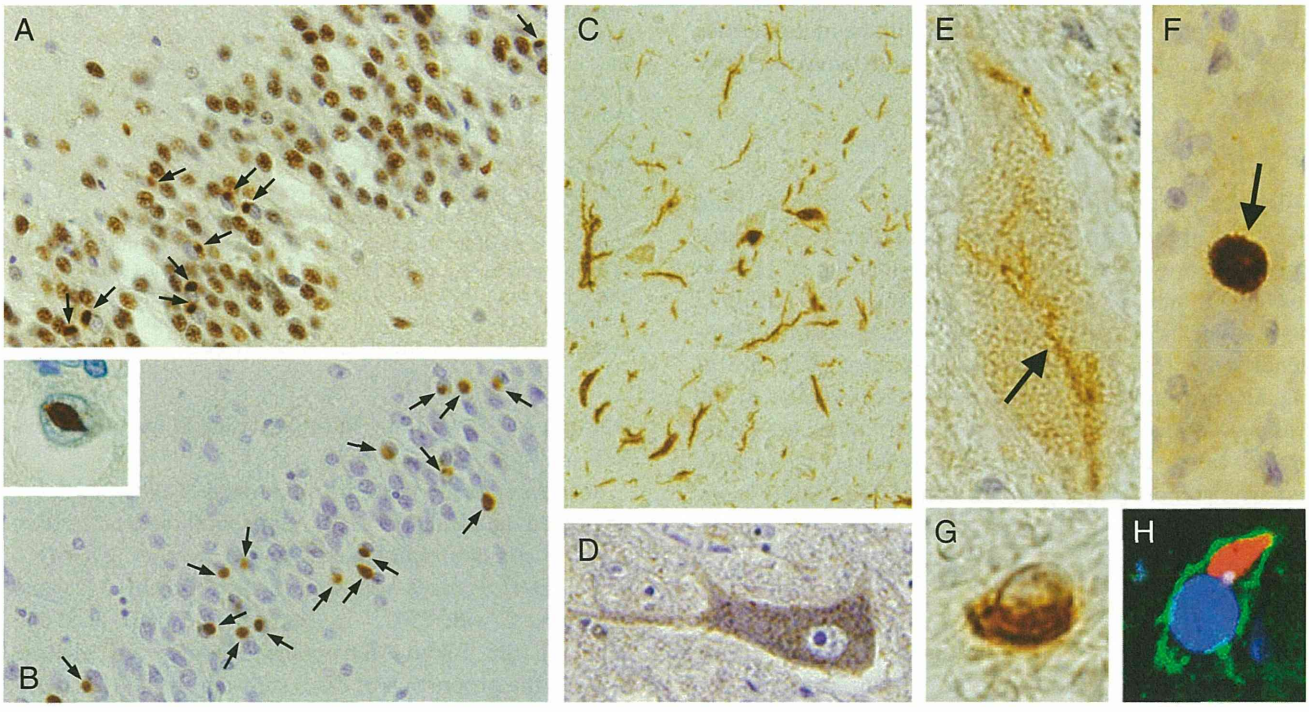

Fig. 1 Neuronal and glial structures immunopositive for trans-activation response (TAR) DNA-binding protein of M_r 43 kDa (TDP-43) in frontotemporal lobar degeneration with TDP-43 pathology (FTLD-TDP) and amyotrophic lateral sclerosis (ALS) A. Dentate gyrus (DG) of the hippocampus of the FTLD-TDP case stained with the commercially available phosphorylation-independent anti-TDP-43 antibody. Both neuronal cytoplasmic inclusions (NCIs) (arrows) and normal neuronal nuclei are immunopositive. B. DG of the FTLD-TDP case stained with the phosphorylated TDP-43-specific antibody (pS409/410). NCIs (arrows) are stained with no nuclear staining. Inset reveals a neuronal intranuclear inclusion with a "cat's eye" shape. C. Dystrophic neurites (DNs) in the frontal cortex of the FTLD-TDP positive for pS409/410. Cytoplasmic granular structures ("preinclusion") stained with the phosphorylation-independent anti-TDP-43 antibody (D), and skein-like inclusion (E), round inclusion (F), and glial cytoplasmic inclusion (G) stained with pS409/410 in the anterior horn of the ALS cases. In double-label immunofluorescence histochemistry using pS409/410 (red in H) and anti-C4d (green in H), the pS409/410-positive inclusion (red) is present around the nucleus of the C4d-positive oligodendrocyte (green).

elongated DNs are generally more widely dispersed throughout the entire cortex compared with the short DNs.[46] Such morphological features of DNs are one of the factors to determine the pathological classification of FTLD-TDP as described below.

Preinclusions (Fig. 1D)

Diffuse or granular cytoplasmic staining of TDP-43 are frequently found in motor neurons of cranial nerve nuclei or spinal cord of ALS and FTLD-TDP cases. They are sometimes referred as preinclusions, and are thought to show a transitional stage in which cytoplasmic TDP-43 remains unassembled or partially assembled into inclusions.[3,35] Mori *et al.* newly found two types of TDP-43 positive neuronal cytoplasmic structures in ALS cases, linear wisps and dot-like inclusions, which may be premature structures of SLIs and round inclusions (RIs), respectively.[47]

SLIs (Fig. 1E) and round inclusions (Fig. 1F)

The SLIs were first reported by Leigh *et al.* as a ubiquitin-positive, skein-like array in anterior horn cells in MND.[32,33] SLIs and spherical or round inclusions in the motoneurons are specific for sporadic ALS cases and found in 100% of them.[34]

GCIs (Fig. 1G,H)

GCIs are immunopositive for TDP-43[1,2,35] (Fig. 1G) and p62,[48] but are mostly negative for ubiquitin.[35,48] In double immunofluorescence staining forphosphorylated TDP-43 (pTDP-43) and a complement protein, C4d, pTDP-43 positive inclusions are often found in C4d-positive oligodendrocytes (Fig. 1H), indicating that most GCIs are of oligodendrocytic origin.[49] They are more frequent in the white as compared with the gray matter.[35] They are found in most cases of TDP-43 proteinopathy to varying degrees, but are the most frequent in ALS and FTLD-MND cases.

Classification of TDP-43 pathology in TDP-43 proteinopathy

Based on cerebral ubiquitin immunohistochemistry, FTLD-TDP was classified into three subtypes by Sampathu *et al.*[31] and Mackenzie *et al.*.[29] Unfortunately, the numbering schemes used in these two systems do not match. Then, Cairns *et al.* drew these two systems together into a unified scheme, and added familial FTLD-TDP with *VCP* mutations.[45] Finally, in 2011, the classification systems were harmonized,[50] and four subtypes were recognized (Fig. 2A): type A had numerous NCIs and short DNs; type B had numerous NCIs with few DNs; type C was characterized by DNs which were often longer and thicker than

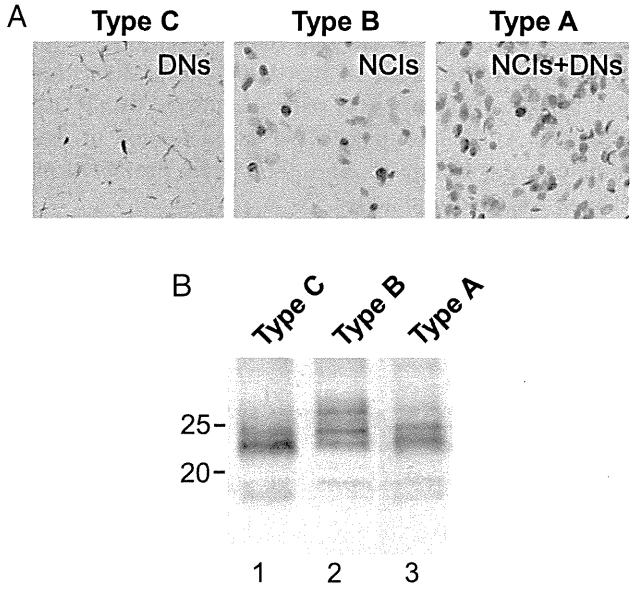

Fig. 2 Pathological and biochemical classification of frontotemporal lobar degeneration with trans-activation response (TAR) DNA-binding protein of M_r 43 kDa (FTLD-TDP). A. FTLD-TDP subtypes A to C. Type A has numerous neuronal cytoplasmic inclusions (NCIs) and short dystrophic neurites (DNs); type B has numerous NCIs; type C is characterized by long and tortuous DNs. B. Representative immunoblots with the phosphorylated TDP-43 specific antibody, pS409/410. The sporadic FTLD-TDP case with type C pathology shows two major bands at 23 and 24 kDa and two minor bands at 18 and 19 kDa (lane 1), while the FTLD-MND (motor neurone disease) case with type B pathology shows three major bands at 23, 24 and 26 kDa and two minor bands at 18 and 19 kDa (lane 2). A 23 kDa band is the most intense in sporadic FTLD-TDP (lane 1), while a 24 kDa band is the most intense in FTLD-MND (lane 2). The band pattern of the case of familial FTLD with *GRN* gene mutation with type A pathology is not distinctive but intermediate between FTLD-TDP and FTLD-MND (lane 3).

DNs in type A, with few NCIs; and type D had numerous NIIs and DNs with few NCIs.

Josephs *et al.* reported the proportion of subtypes based on pooled data from four large clinicopathological studies: the most common subtype was type A accounting for 41% of FTLD-TDP cases, followed by type B with 34% and then type C with 25%.[51] In 29 cases of FTLD-TDP collected in the Tokyo Metropolitan Institute of Medical Science, the proportion of cases showing types A, B and C was 17%, 48% and 34%, respectively.[52] The lower frequency of type A in our series may be partly explained by the low frequency of familial cases of FTLD in Japan.[52–54]

ALS is pathologically classified into two types based on the TDP-43 neuronal distribution pattern by Nishihira *et al.*[36] Type 2 is distinguished from type 1 by the TDP-43-positive NCIs in the frontotemporal cortex, hippocampal formation, neostriatum and substantia nigra, and is significantly associated with dementia.

Association between clinical and pathological subtypes of TDP-43 proteinopathy

FTLD is clinically subclassified into three categories: behavioral variant frontotemporal dementia (bvFTD), semantic dementia (SD) and progressive non-fluent aphasia (PNFA). The strong correlations between the subtype of TDP-43 pathology and clinical phenotype are indicated, although a strict one-to-one relationship is lacking.[55] Type C is associated with SD, type B with FTLD with motor neuron disease (MND) or clinical sign of MND, and type A with PNFA.[29,51,52,56,57] bvFTD is not as strongly associated with any one type.[51,52,56] As for the correlation between genetics and subtypes, *GRN* mutations are associated with type A, *C9OFR72* with types A and B, and *VCP* with type D.

UNDERLYING MECHANISM

Phosphorylation and truncation of TDP-43 accumulated in diseased brains

There are multiple potential phosphorylation sites within human TDP-43, including 41 serine (Ser), 15 threonine (Thr) and seven tyrosine (Tyr) residues. In order to identify the critical phosphorylation sites of TDP-43 accumulated in diseased brains, we raised antibodies against 39 different synthetic phosphopeptides, representing 36 out of 63 candidate phosphorylation sites.[58] The major strategy was to choose Ser and Thr residues that cover known protein kinase consensus phosphorylation motifs, including R-X-pSer/Thr for protein kinase A (PKA), pSer/Thr-X-X-Ser/Thr for CK1, pSer/Thr-X-X-E/D for CK2, pSer/Thr-X-X-X-Ser for GSK3 and CK1. Additionally, Ser/Thr residues in the C-terminal region of TDP-43 were chosen because they are analogous to abnormal phosphorylation sites found in tau or α-synuclein.

Of the generated antibodies, pS379, pS403/404, pS409, pS410 and pS409/410 stained the inclusions in immunohistochemistry, and abnormal TDP-43 species on immunoblot, in FTLD-TDP and ALS cases. These results suggest that at least five sites on TDP-43 are phosphorylated in subjects with FTLD-TDP and ALS, and that abnormal phosphorylation takes place mainly near the C-terminal region of TDP-43. This is similar to tauopathies and synucleinopathies, where multiple Ser residues in the C-terminal region in tau and Ser129 in α-synuclein, are abnormally phosphorylated.[59,60] Phosphorylation of Ser409/410 of TDP-43 accumulated in diseased brains was confirmed by other groups.[61,62]

In contrast to the commercially obtained phosphorylation-independent anti-TDP-43 antibody, which labels both abnormal structures and normal nuclei (Fig. 1A), pTDP-43 specific antibodies recognize only abnormal structures, including NCIs (Fig. 1B), NIIs (Fig. 1B, inset), DNs (Fig. 1C), SLIs (Fig. 1E), round inclusions (Fig. 1F) and GCIs (Fig. 1G,H). These results suggest that all of the inclusion types previously described in FTLD-TDP and ALS contain pTDP-43.

Immunoblot analyses of sarkosyl-insoluble fractions with pTDP-43-specific antibodies reveal a single band at 45 kDa, several smaller fragments at ~25 kDa and indistinct smears in FTLD-TDP and ALS cases but not in controls. The intensity of the ~25 kDa fragments tends to be greater than that of the 45 kDa band in FTLD-TDP and in ALS.

Biochemical features of pathological subtypes of FTLD-TDP

To investigate the biochemical basis of the different TDP-43 pathological subtypes (type A-C), we carefully compared the results of immunoblots of the sarkosyl-insoluble fractions from the cerebral cortex of cases with sporadic FTLD-TDP (type C), FTLD-MND (type B), ALS (type B) and familial FTLD with *GRN* mutations (type A), using pTDP-43-specific antibodies. The results showed that there is a close relationship between the pathological subtypes and the immunoblot pattern of the 18~26 kDa C-terminal fragments of pTDP-43[58] (Fig. 2). These results parallel our earlier findings of differing C-terminal tau fragments in progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD), despite identical composition of tau isoforms.[63]

Prion-like properties of pathological TDP-43

Prion-like propagation of aggregated proteins in neurodegenerative diseases has been well established.[64–73] In prion diseases, the banding patterns of protease-resistant prion protein extracted from diseased brains have been reported to differ among the subtypes.[74] For instance, protease-resistant prion protein extracted from cases with new-variant Creutzfeldt–Jakob disease showed a different and characteristic pattern from that in cases with sporadic Creutzfeldt–Jakob disease. Protease-treated prion protein species are thought to have different mobilities because of different conformations. These findings in prion disease suggest that the different band patterns in TDP-43 proteinopathies may represent different conformations of abnormal TDP-43 in the diseases. Indeed, Tsuji *et al.* found that on chymotrypsin treatment of Sarkosyl-insoluble pellets extracted from brains of patients with FTLD-TDP, the patterns of multiple protease-resistant bands of TDP-43 at 16–25 kDa differed among three subtypes of FTLD-TDP.[75] In addition, there is no difference between the band pattern of TDP-43 C-terminal fragments in cortex

Table 1 Comparison between FTLD-PLS and FTLD-ALS[52]

	FTLD-PLS	FTLD-ALS
Clinical features		
LMN signs	Absent	Present
UMN signs	Present	Present
Pathological features		
CST degeneration	Severe	Mild
LMN loss	Absent (or minimal)	Evident
TDP-43 positive NCIs in LMN	Absent (or rare)	Present
TDP-43 positive RSs in the neuropil of SC	Present	Absent
Bunina bodies	Absent	Present
Common subtype of TDP-43 pathology	Type C	Type B

CST, corticospinal tract; FTLD-ALS, frontotemporal lobar degeneration with amyotrophic lateral sclerosis; FTLD-PLS, frontotemporal lobar degeneration with primary lateral sclerosis; LMN, lower motor neuron; NCIs, neuronal cytoplasmic inclusions; RSs, round structures; SC, spinal cord; UMN, upper motor neuron.

and that in spinal cord in ALS cases, suggesting that abnormal proteins produced in cells is transferred to different regions and propagated.[75,76]

Furthermore, using the cell culture system, Nonaka *et al.* showed that insoluble TDP-43 aggregates in brains of ALS and FTLD-TDP patients have prion-like properties. They showed that insoluble TDP-43 extracted from brains of each subtype of FTLD-TDP (types A, B or C) cases functioned as seeds for TDP-43 aggregation in cultured cells.[77] Interestingly, the band patterns of TDP-43 C-terminal fragments in the insoluble fraction of cells expressing TDP-43 in the presence of each type of seed were different from each other, but quite similar to that of insoluble TDP-43 prepared as seeds from the corresponding patients. These results suggest that seed-dependent TDP-43 aggregation occurs in a self-templating manner like prion aggregation. They also showed prion-like properties of abnormal TDP-43, including stability to heat and proteinases, and cell-to-cell transmission at least partly via exosomes.[77]

CURRENT AMBIGUITIES AND PERSPECTIVE

FTLD-PLS

Recently, the presence of FTLD-TDP showing corticospinal tract (CST) degeneration but lacking lower motor neuron (LMN) loss was reported[78–80] and the term primary lateral sclerosis (PLS) is used to distinguish MND of these cases from ALS.[78,79] Of 29 FTLD-TDP cases collected in Tokyo Metropolitan Institute of Medical Science, we identified 10 FTLD with PLS (FTLD-PLS) cases.[52]

Clinically, the first symptoms were bvFTD in five cases, SD in three cases and PNFA in one case. The upper motor neuron (UMN) signs, including hyperreflexia, spasticity, Babinski's sign, paralysis and ankle clonus, were recorded in six cases, and five of six cases developed UMN signs in the middle or late stage of the disease. There were no LMN signs in any case. In terms of the TDP-43 pathology, six cases showed type C, three cases type A, and one case type B. In four cases in which the spinal cord was available, there were no skein-like inclusions or round inclusions in all cases, but TDP-43 positive round structures in the neuropil of the anterior horn were found in two cases with type C TDP-43 pathology. The clinicopathological comparison between FTLD-PLS and FTLD-ALS is summarized in Table 1. These results suggest that FTLD-PLS may be a significant clinicopathological entity distinct from FTLD-ALS.[52] The different band pattern of C-terminal fragments of TDP-43 on immunoblotting of sarkosyl-insoluble fraction between FTLD-PLS and ALS, reported by Kosaka *et al.*,[81] further supports our notion. Recently, Josephs *et al.* reported that of three subtypes of FTLD, CST was associated mostly with FTLD with type C pathology with predominant atrophy of the right temporal lobe.[82]

The significance of TDP-43 pathology in other neurodegenerative disorders

Immunohistochemical examination, using commercially available phosphorylation-independent anti-TDP-43 antibodies, had demonstrated abnormal intracellular accumulation of TDP-43 in neurodegenerative disorders other than FTLD-TDP and ALS, including ALS/parkinsonism-dementia complex of Guam,[83–85] Alzheimer's disease (AD),[86–90] dementia with Lewy bodies (DLB),[87,91] Pick's disease,[2,92,93] hippocampal sclerosis,[86] CBD[90] and PSP.[94] By immunohistochemical and biochemical analyses using pTDP-43-specific antibodies, we found a high frequency of pTDP-43 pathology in cases of AD (36–56%) (Fig. 3A,C), DLB (53–60%) (Fig. 3B,D), argyrophilic grain disease (AGD) (60%) (Fig. 3E), Huntington's disease (100%) and a case of familial British dementia.[95–98]

The pathological significance and mechanism of such frequent co-occurrence of diverse protein aggregates are

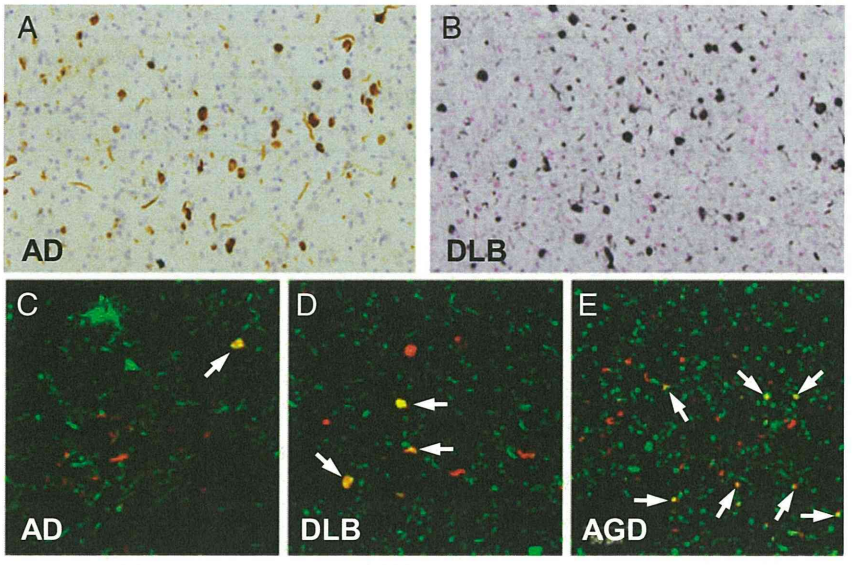

Fig. 3 Trans-activation response (TAR) DNA-binding protein of M_r 43 kDa (TDP-43)-positive structures in other neurodegenerative disorders. Neuronal cytoplasmic inclusions (NCIs) and short dystrophic neurites (DNs) stained with the pTDP-43-specific antibody (pS403/404) in the temporal cortex of the Alzheimer's disease (AD) case (A) and the dementia with Lewy bodies (DLB) case (B). (C–E) Double-label immunofluorescence histochemistry of the temporal cortex of AD (C) and DLB (D) and of the amygdala of argyrophilic grain disease (AGD) (E). The green fluorescence reveals the immunoreactivity for phosphorylated tau (AT8) in C and E, and that for phosphorylated alpha-synuclein in D, while the red fluorescence represents the immunopositivity for pS403/404 in C–E. Arrows indicate the colocalization of tau and pTDP-43 in C and E, and that of alpha-synuclein and pTDP-43 in D.

still unclear. A higher Braak NFT stage in the TDP-43 positive patients than in the TDP-43 negative ones was found in DLB+AD cases[91] and in our study of AD cases.[95] We also reported parallel distribution of TDP-43 positive structures and tau positive grains and higher AGD stages in cases with TDP-43 immunoreactivity than in those without TDP-43 immunoreactivity in AGD.[97] Double-label immunofluorescence microscopy reveals partial colocalization of tau and TDP-43 in AD, DLB, AGD, Guamanian PDC and CBD[83,84,86,87,90,91,97] or of α-synuclein and TDP-43 in DLB.[87,91,95] These findings suggest that there may be common factors or mechanisms that affect the conformation or modification of these proteins, leading to their intracellular accumulation.

Regarding the typing of TDP-43 pathology, neocortical TDP-43 pathology in AD and DLB corresponded to type A.[90,95,99] Immunoblot analyses of the sarkosyl insoluble fraction from AD and DLB cases with neocortical TDP-43 pathology also showed that the band pattern of these CTFs in AD and DLB corresponded to that of type A[58]. These results suggest that the morphological and biochemical features of TDP-43 pathology are common between AD or DLB and a specific subtype of FTLD-TDP. Since all FTLD-TDP cases with *GRN* mutations show type A pathology,[45] there may be genetic factors, such as mutations or genetic variants of *GRN* underlying the co-occurrence of abnormal deposition of TDP-43, tau and α-synuclein. Indeed, recently, *GRN* loss-of-function mutation has been confirmed in patients clinically diagnosed with AD[100–105] and Parkinson's disease.[106] The association between rs5848 variant in the 3′ untranslated region of *GRN* and risk of AD has been reported in a Taiwanese population,[107] suggesting that homozygous TT genotype accentuates the risk of AD. These findings suggest that PGRN reduction may induce both TDP-43 pathology and AD pathology.

Cell death and TDP-43 pathology

A report using a cell culture system showed that intracellular aggregate formation of TDP-43 induced cell death.[77] In brains of FTLD and ALS cases, basically, the occurrence of TDP-43-positive neuronal structures is related to degenerative changes.[36] However, the issue of the relation between the formation of TDP-43-positive inclusions and cell death may not be straightforward, since neuronal loss was not evident in the hippocampal granule cells[36] and the neostriatum[108] where TDP-43-positive structures were present in ALS cases. The reason for such a discrepancy between the results of the cell culture experiments and the findings of diseased brains should be discussed as a future issue.

CONCLUSION

The relevance of the pathological classification of TDP-43 proteinopathy is supported by clinical, biochemical and genetic correlations, although there is still highly significant heterogeneity in cases with type A pathology (Table 2). The results of the biochemical analyses of the diseased brains and the cellular models suggest that different strains of TDP-43 with different conformations may determine the clinicopathological phenotypes of TDP-43 proteinopathy, like prion disease. Detecting each TDP-43 strain in biological fluids may be useful for the differential diagnosis of TDP-43 proteinopathy. Furthermore, elucidating the mechanism of the conformational changes leading to the formation of multiple TDP-43 strains may be important for developing disease-modifying therapy for these diseases.

Table 2 Clinical, pathological and genetic associations of TDP-43 proteinopathy and other neurodegenerative disorders

Pathological subtype	Associated genes	Clinical phenotypes					Other diseases
		bvFTD	PNFA	SD		MND	
				Semantic aphasia	Prosopagnosia		
A	GRN, C9ORF72	+	+			PLS, ALS	AD, DLB
B	C9ORF72	+	+			ALS	
C				+			
					+	PLS	
D	VCP	+				ALS	

ALS, amyotrophic lateral sclerosis; AD, Alzheimer's disease; bvFTD, behavioral variant frontotemporal dementia; DLB, dementia with Lewy bodies; MND, motor neuron disease; PLS, primary lateral sclerosis; PNFA, progressive non-fluent aphasia; SD, semantic dementia.

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Distinct pathways leading to TDP-43-induced cellular dysfunctions

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TAR DNA-binding protein of 43 kDa (TDP-43) is the major component protein of inclusions found in brains of patients with amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD-TDP). However, the molecular mechanisms by which TDP-43 causes neuronal dysfunction and death remain unknown. Here, we report distinct cytotoxic effects of full-length TDP-43 (FL-TDP) and its C-terminal fragment (CTF) in SH-SY5Y cells. When FL-TDP was overexpressed in the cells using a lentiviral system, exogenous TDP-43, like endogenous TDP-43, was expressed mainly in nuclei of cells without any intracellular inclusions. However, these cells showed striking cell death, caspase activation and growth arrest at G2/M phase, indicating that even simple overexpression of TDP-43 induces cellular dysfunctions leading to apoptosis. On the other hand, cells expressing TDP-43 CTF showed cytoplasmic aggregates but without significant cell death, compared with cells expressing FL-TDP. Confocal microscopic analyses revealed that RNA polymerase II (RNA pol II) and several transcription factors, such as specificity protein 1 and cAMP-response-element-binding protein, were co-localized with the aggregates of TDP-43 CTF, suggesting that sequestration of these factors into TDP-43 aggregates caused transcriptional dysregulation. Indeed, accumulation of RNA pol II at TDP-43 inclusions was detected in brains of patients with FTLD-TDP. Furthermore, apoptosis was not observed in affected neurons of FTLD-TDP brains containing phosphorylated and aggregated TDP-43 pathology. Our results suggest that different pathways of TDP-43-induced cellular dysfunction may contribute to the degeneration cascades involved in the onset of ALS and FTLD-TDP.

INTRODUCTION

TAR DNA-binding protein of 43 kDa (TDP-43) has been identified as a major component protein of the ubiquitinated inclusions characteristic of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration with ubiquitin-positive inclusions (FTLD-U or FTLD-TDP) (1,2). TDP-43 is a ubiquitously expressed nuclear protein and is implicated in exon splicing, gene transcription, regulation of mRNA stability and biosynthesis and formation of nuclear bodies (3–7). It is a 414-amino acid protein with two highly conserved RNA recognition motifs (RRM1 and RRM2) and a glycine-rich region mediating protein–protein interactions at the C-terminus (8–11). In TDP-43 proteinopathy, pathological TDP-43 is abnormally phosphorylated, ubiquitinated and N-terminally cleaved to generate C-terminal fragments (CTFs) (1,12,13).

As autosomal-dominant missense mutations in the *TARDBP* gene were identified in patients with ALS or FTLD-TDP, toxic gain of function of TDP-43 may be related to neuronal degeneration. However, in most cases of TDP-43 proteinopathy, no *TARDBP* mutations are identified, suggesting that wild-type TDP-43 itself is central to the disease cascade. A 2-fold increase in total *TARDBP* mRNA was reported in a 3′-untranslated region variant carrier (14), which suggests that just an increased level of

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