Received: 30 July 2021 | Revised: 18 November 2021 | Accepted: 12 December 2021

DOI: 10.1111/nan.12786

ORIGINAL ARTICLE

Neuropathology and Applied Neurobiology
JOURNAL OF THE BRITISH NEUROPATHOLOGICAL SOCIETY

WILEY

Brain TDP-43 pathology in corticobasal degeneration: Topographical correlation with neuronal loss

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Funding information
Grants-in-Aid, Japan Agency for Medical Research and Development, Grant/Award Numbers: JP21ek0109545, JP21dk0207045; JSPS Grants-in-aid for Scientific Research, Grant/Award Numbers: 19H05559, 19H01061, 19K07972; Grants-in Aid from the Research Committee of CNS Degenerative Diseases, Research on Policy Planning and Evaluation for Rare and Intractable Diseases, Health, Labor and Welfare Sciences Research Grants, the Ministry of Health, Labour and Welfare, Japan

Abstract

Aims: Neuronal and glial inclusions comprising transactive response DNA-binding protein of 43 kDa (TDP-43) have been identified in the brains of patients with corticobasal degeneration (CBD), and a possible correlation between the presence of these inclusions and clinical phenotypes has been speculated. However, the significance of TDP-43 pathology in the pathomechanism of CBD has remained unclear. Here, we investigated the topographical relationship between TDP-43 inclusions and neuronal loss in CBD.

Methods: We estimated semi-quantitatively neuronal loss and TDP-43 pathology in the form of neuronal cytoplasmic inclusions (NCIs), astrocytic inclusions (AIs), oligodendroglial cytoplasmic inclusions (GCIs), and dystrophic neurites in 22 CNS regions in 10 patients with CBD. Then, the degree of correlation between the severity of neuronal loss and the quantity of each type of TDP-43 inclusion was assessed. We also investigated tau pathology in a similar manner.

Results: TDP-43 pathology was evident in nine patients. The putamen and globus pallidus were the regions most frequently affected (80%). NCIs were the most prominent form, and their quantity was significantly correlated with the severity of neuronal loss in more than half of the regions examined. The quantities of TDP-43 NCIs and tau NCIs were correlated in only a few regions. The number of regions where the quantities of TDP-43 AIs and GCIs were correlated with the severity of neuronal loss was apparently small in comparison with that of NCIs.

Conclusions: TDP-43 alterations in neurons, not closely associated with tau pathology, may be involved in the pathomechanism underlying neuronal loss in CBD.

There was a significant topographical correlation between neuronal cytoplasmic aggregation of TDP-43 and neuronal loss in CBD, suggesting that TDP-43 protein aberration might be associated with neuronal degeneration in CBD. There was no close correlation between the burden of TDP-43 and that of tau in neurons.

KEYWORDS
corticobasal degeneration, corticobasal syndrome, tau, TDP-43

INTRODUCTION

Corticobasal degeneration (CBD) is a representative disorder of four-repeat tauopathy. It is characterised histologically by widespread and abundant deposition of hyperphosphorylated four-repeat tau in neurons and glia, in the form of astrocytic plaques, coiled bodies or neuropil threads in both the grey and white matter [1, 2].

As well as tau-immunolabelled inclusions (tau inclusions), inclusions immunolabelled for transactive response DNA-binding protein of 43 kDa (TDP-43) (TDP-43 inclusions) have been observed in neurons and glia of patients with CBD [3–9]. Previous post-mortem studies have revealed that the frequency of TDP-43 inclusions in CBD appears to be much higher than in progressive supranuclear palsy (PSP) and that the distribution of the inclusions in CBD might differ from that in frontotemporal lobar degeneration (FTLD)-TDP and limbic predominant age-related TDP-43 encephalopathy (LATE) [7, 9]. In patients with CBD, the clinical picture suggests that TDP-43 pathology could be associated with PSP syndrome (PSPS) [7] or Richardson syndrome [9]. However, the relationship of TDP-43 pathology with the histopathological features of CBD has remained unclear.

TDP-43 is a multifunctional nuclear protein involved in the regulation of transcription, RNA splicing, and translation [10–12]. Under normal conditions, TDP-43 is localised in the nucleus, but in pathologic conditions, it aberrantly localises and aggregates in the cytoplasm [13, 14]. It is well known that the presence of TDP-43 inclusions is a hallmark of amyotrophic lateral sclerosis (ALS) and FTLD-TDP [15]. However, recent pathological studies have identified TDP-43 inclusions in the brains of patients with other neurodegenerative disorders, including Alzheimer's disease (AD), dementia with Lewy bodies (DLB) and Huntington's disease [3, 8, 16–18]. This suggests the possible involvement of TDP-43 in the cellular pathomechanisms underlying a number of neurodegenerative disorders characterised according to the various proteins responsible, including tau, α-synuclein and polyglutamine.

In the present study, we aimed to determine whether TDP-43 pathology correlates with neuronal loss in patients with CBD through semi-quantitative and topographical evaluation of TDP-43 inclusions in both neurons and glia observed in sections cut from various brain regions. We also estimated the severity of neuronal loss, and the quantity of tau inclusions in these regions, and assessed the degree of correlation between these factors.

Key points

- TDP-43 inclusions were observed in the CNS in 9 of 10 patients with CBD.
- Neuronal cytoplasmic inclusions were the most prominent form of TDP-43 inclusion.
- There was a significant topographical correlation between neuronal cytoplasmic aggregation of TDP-43 and neuronal loss in CBD.
- There was no close correlation between the burden of TDP-43 and that of tau in neurons.

MATERIALS AND METHODS

Subjects

We retrieved 10 consecutive patients with pathologically proven CBD (6 male and 4 female; age [mean ± standard deviation] 71.4 ± 7.2 years, range 65–87 years) from our institutional autopsy files covering the period between 1990 and 2017. All the patients were of Japanese ancestry, and their clinical information was obtained retrospectively by reviewing their medical records. The clinicopathological profiles are summarised in Table 1. The clinical diagnosis at the time of autopsy was CBD or corticobasal syndrome (CBS) ($n = 6$), PSP or PSPS ($n = 2$), DLB ($n = 1$) and frontotemporal dementia ($n = 1$). At present, since four clinical phenotypes in CBD—that is, CBS, frontal behavioural-spatial syndrome (FBSS), non-fluent/agrammatic variant of primary progressive aphasia and PSPS—are recognised [19], we re-evaluated the clinical phenotypes of all individuals retrospectively. As a result, six and four patients were categorised as having CBS (one probable and five possible) and PSPS, respectively; one patient with CBS and another with PSPS also met the criteria for FBSS. To compare the following data between patients with CBS and PSPS, we performed Fisher's exact test for comparison of categorical data and Mann-Whitney U test for non-parametric analysis of continuous variables. There was no significant difference in gender ratio (male: female, 5:1 vs. 1:3, $p = 0.19$), or age at onset (63.7 ± 5.5 vs. 64.5 ± 5.8, $p = 0.92$) between these two groups, while the disease duration was significantly longer in patients with CBS than in those with PSPS (9.2 ± 4.0 vs. 4.8 ± 1.0 years, $P = 0.03$). Brain weight did not differ significantly between the groups (1,018 ± 87 vs. 1093 ± 197 g, $P = 0.39$). In accordance with the 'ABC' score for AD neuropathologic change [20], three and seven patients corresponded to 'Not' and 'Low', respectively. Lewy bodies and Lewy neurites were observed in two patients ('brainstem predominant' type in one and 'amygdala predominant' type in the other, in accordance with the DLB consensus guidelines [21]). Argyrophilic grains were observed in seven patients. Hippocampal sclerosis [22] was not evident in any of the patients. Written informed consent for autopsy, including the use of tissue for research purposes, was obtained from the next of kin. The present study was approved by the Institutional Review Board of Niigata University School of Medicine, Japan.

Histology and immunohistochemistry

Multiple formalin-fixed, paraffin-embedded CNS tissue blocks from the patients were available for this study. Histological examinations

TABLE 1 Clinicopathologic characteristics

Patients	1	2	3	4	5	6	7	8	9	10
Clinical phenotype	CBS[a]	CBS[a,b]	CBS[a]	CBS[a]	CBS[a]	CBS[a]	PSPS[b]	PSPS	PSPS	PSPS
Sex	M	M	F	M	M	M	F	F	M	F
Age at onset (year)	74	62	63	64	61	58	59	72	61	66
Disease duration (year)	13	5	15	7	6	9	6	5	4	4
Brain weight (g)	1,020	985	885	1,010	1,150	1,060	850	1,120	1,330	1,070
TDP-43 inclusions	+	+	+	+	+	−	+	+	+	+
ABC score[20]	A1B2C0	A0B1C0	A0B2C0	A1B1C0	A0B1C0	A1B0C0	A1B1C2	A1B1C0	A1B1C0	A1B1C2
Argyrophilic grains	−	−	+	+	−	+	+	+	+	+
Lewy bodies[21]	−	+[c]	−	+[c]	−	−	−	−	−	−

Abbreviations: CBS, corticobasal syndrome; F, female; M, male; PSPS, progressive supranuclear palsy syndrome.
[a]Patient 1 corresponded to probable, and Patients 2 to 6 corresponded to possible CBS according to Armstrong et al. [19].
[b]Patients 2 and 7 also developed features consistent with those of frontal behavioural-spatial syndrome.
[c]Patient 2 showed amygdala-predominant, and Patient 4 brainstem-predominant Lewy-related pathology.

were performed on 4-μm-thick sections cut from various brain and spinal cord regions listed in Table 2, using haematoxylin-eosin (HE) and Klüver-Barrera staining and Gallyas-Braak silver impregnation. Immunohistochemistry was performed as described previously [23, 24] using mouse monoclonal antibodies against phosphorylated TDP-43 (pTDP-43; phospho Ser409/410; Cosmo Bio, Tokyo, Japan), hyperphosphorylated tau (AT8; Innogenetics, Ghent, Belgium) and phosphorylated α-synuclein (pSyn#64; Wako, Osaka, Japan). Bound antibodies were visualised by the peroxidase-polymer-based method using a Histofine Simple Stain MAX-PO kit (Nichirei Biosciences, Tokyo, Japan) with diaminobenzidine as the chromogen. Counterstaining was conducted with Mayer's haematoxylin. The histopathologic diagnosis of CBD was confirmed on the basis of neuronal loss in the focal cortical regions and in the substantia nigra, and tau immunohistochemistry to reveal neurofibrillary tangles, astrocytic plaques, coiled bodies and extensive neuropil threads in the cerebral cortex, white matter and basal ganglia [1].

Semi-quantitative analysis

For all CNS regions, 4-point semiquantitative analysis of neuronal loss (0, none; 1, mild; 2, moderate; 3, severe) was performed using HE sections. The quantities of TDP-43 inclusions (Figure 1) and tau inclusions (Figure 2), in the form of neuronal cytoplasmic inclusions (NCIs), astrocytic inclusions (AIs), or oligodendroglial cytoplasmic inclusions (GCIs), were assessed semi-quantitatively as an 'appearance score' (0, absent; 1, sparse; 2, moderate; 3, frequent). TDP-43-immunolabelled dystrophic neurites (DNs) and tau-immunolabelled threads were assessed similarly in terms of a 4-point appearance score. The median scores for neuronal loss, the proportion of patients possessing each form of TDP-43 inclusion and the median appearance score for each form of TDP-43 inclusion in each region were indicated using colour coding (Table 2). Then, the correlation between the neuronal loss score and the appearance score for each inclusion was assessed. We also observed a small number of TDP-43-immunolabelled neuronal intranuclear inclusions in several regions but did not include them in this study because of their rarity. The analysis was performed by one of the authors (M.S.) and reviewed by another investigator (M.T.) to ensure consistency.

Double-labelling immunofluorescence

Double-labelling immunofluorescence analyses were performed on sections of the motor cortex and putamen using a rabbit polyclonal antibody against phospho Ser409 TDP-43 (Cosmo Bio) with a mouse monoclonal antibody against hyperphosphorylated tau (AT8), and on sections of the frontal cortex using a rabbit polyclonal antibody against phospho Ser409 TDP-43 with a mouse monoclonal antibody against aldehyde dehydrogenase 1 family member L1 (ALDH1L1; 7G8; eBioscience, San Diego, CA, USA). The second antibodies used were Alexa Fluor 488 goat anti-rabbit IgG and Alexa Fluor 568 goat anti-mouse IgG (Molecular Probes, Eugene, OR, USA). The sections were treated with an Autofluorescence Eliminator Reagent (Millipore, Billerica, MA, USA), mounted under glass coverslips using VectaShield mounting medium with 4,6-diamidino-2-phenylindole (DAPI) nuclear stain (Vector Laboratories, Burlingame, CA, USA) and analysed using a confocal laser scanning microscope (LSM700, Carl Zeiss, Oberkochen, Germany).

Statistical analysis

Mann-Whitney *U* test was used for non-parametric analysis of semi-quantitative data for the severity of neuronal loss and the appearance scores for each form of TDP-43 inclusion in each region between patients with CBS and those with PSPS, and in the

TABLE 2 Regional severity of neuronal loss and quantity of TDP-43 inclusions

CNS regions	Neuronal loss score	TDP-43 inclusions								
	median (25th, 75th)	Patients harbouring inclusions [% (n)]					Appearance score [median (25th, 75th)]			
		Overall*	NCIs	AIs	GCIs	DNs	NCIs	AIs	GCIs	DNs
Frontal cortex	2 (1.25, 2)	50 (5/10)	50 (5/10)	50 (5/10)	50 (5/10)	50 (5/10)	0.5 (0, 1)	0.5 (0, 1)	0.5 (0, 1)	0.5 (0, 1)
Frontal white matter	na	40 (4/10)	na	40 (4/10)	40 (4/10)	40 (4/10)	na	0 (0, 1)	0 (0, 1)	0 (0, 1)
Motor cortex	3 (2, 3)	70 (7/10)	50 (5/10)	30 (3/10)	60 (6/10)	70 (7/10)	0.5 (0, 1)	0 (0, 1.5)	1 (0, 1.75)	1 (0.25, 1.75)
Putamen	3 (2, 3)	80 (8/10)	80 (8/10)	70 (7/10)	60 (6/10)	80 (8/10)	2 (1, 3)	1 (0.25, 1.75)	1 (0, 1)	1 (1, 1)
Globus pallidus	2 (1, 3)	80 (8/10)	80 (8/10)	30 (3/10)	80 (8/10)	80 (8/10)	2 (1, 2)	0 (0, 0.75)	1 (1, 1)	1 (1, 1)
Thalamus	1 (0.25, 2.75)	70 (7/10)	70 (7/10)	30 (3/10)	60 (6/10)	60 (6/10)	1 (0.25, 1)	0 (0, 0.75)	1 (0, 2)	1 (0, 1)
Subthalamic nucleus	2.5 (1.75, 3)	75 (6/8)	75 (6/8)	50 (4/8)	75 (6/8)	75 (6/8)	1.5 (0.75, 2)	0.5 (0, 1.25)	1 (0.75, 1)	1 (0.75, 1)
Amygdala	2 (1, 2)	70 (7/10)	70 (7/10)	70 (7/10)	50 (5/10)	70 (7/10)	2 (0.25, 2)	1 (0.25, 2)	0.5 (0, 1)	1 (0.25, 1)
Ammon CA1	1 (1, 2)	70 (7/10)	60 (6/10)	30 (3/10)	70 (7/10)	60 (6/10)	1 (0, 2)	0 (0, 0.75)	1 (0.25, 1)	1 (0, 2)
Dentate gyrus	1.5 (0.25, 2)	60 (6/10)	60 (6/10)	0 (0/10)	60 (6/10)	40 (4/10)	1 (0, 1)	0 (0, 0)	1 (0, 1)	0 (0, 1)
Midbrain tegmentum	2.5 (1.25, 3)	70 (7/10)	60 (6/10)	60 (6/10)	60 (6/10)	70 (7/10)	2 (0, 2)	1 (0, 2)	1 (0, 1)	1 (0.25, 1.75)
Superior colliculus	2 (1, 2)	60 (6/10)	60 (6/10)	50 (5/10)	60 (6/10)	60 (6/10)	1.5 (0, 2)	0.5 (0, 1)	1 (0, 2)	1 (0, 1.75)
Substantia nigra	2 (2, 3)	60 (6/10)	60 (6/10)	30 (3/10)	60 (6/10)	60 (6/10)	1.5 (0, 2)	0 (0, 0.75)	1 (0, 1)	1 (0, 1.75)
Pontine tegmentum	3 (2, 3)	70 (7/10)	70 (7/10)	20 (2/10)	70 (7/10)	70 (7/10)	1 (0.25, 2)	0 (0, 0)	1 (0.25, 1.75)	1 (0.25, 1.75)
Pontine nucleus	0 (0, 0.75)	60 (6/10)	50 (5/10)	10 (1/10)	50 (5/10)	60 (6/10)	0.5 (0, 1)	0 (0, 0)	0.5 (0, 1)	1 (0, 1)
Medullary tegmentum	2 (2, 2)	67 (6/9)	56 (5/9)	0 (0/9)	67 (6/9)	67 (6/9)	1 (0, 1)	0, (0, 0)	1 (0, 1)	1 (0, 1)
Inferior olivary nucleus	1 (1, 1)	60 (6/10)	60 (6/10)	40 (4/10)	60 (6/10)	60 (6/10)	1 (0, 2)	0 (0, 1)	1, (0, 1)	1 (0, 1)
Cerebellar cortex	1 (0, 1)	20 (2/10)	0 (0/10)	0 (0/10)	20 (2/10)	10 (1/10)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
Cerebellar white matter	na	40 (4/10)	na	0 (0/10)	40 (4/10)	30 (3/10)	na	0 (0, 0)	0 (0, 1)	0 (0, 0.75)
Dentate nucleus	2 (1, 3)	40 (4/10)	40 (4/10)	0 (0/10)	40 (4/10)	40 (4/10)	0 (0, 1)	0 (0, 0)	0 (0, 1)	0 (0, 1)
Anterior horns (C)	1 (1, 1)	50 (5/10)	10 (1/10)	0 (0/10)	30 (3/10)	50 (5/10)	0 (0, 0)	0 (0, 0)	0 (0, 0.75)	0.5 (0, 1)
Anterior horns (L)	0 (0, 1)	22 (2/9)	11 (1/9)	0 (0/9)	11 (1/9)	22 (2/9)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)

Colour indication

Neuronal loss score		Proportion of patients harbouring TDP-43 inclusions		Appearance score for TDP-43 inclusions	
	Severe (Median = 3)		High (70 ≤ Proportion)		High (1.5 ≤ Median)
	Intermediate (2 ≤ Median < 3)		Intermediate (50 ≤ Proportion < 70)		Intermediate (Median = 1)
	Mild (1 ≤ Median < 2)		Low (10 ≤ Proportion < 50)		Low (Median = 0.5)
	None (Median < 1)		None (Proportion = 0)		None (Median = 0)

Abbreviations: AIs, astrocytic inclusions; C, cervical; DNs, dystrophic neurites; GCIs, glial cytoplasmic inclusions; L, lumbar; na, not applicable; NCIs, neuronal cytoplasmic inclusions.
*Percentages of patients harbouring at least one form of TDP-43 inclusion (NCIs, AIs, GCIs or DNs).

amygdala between patients with and without argyrophilic grains. Goodman and Kruskal's gamma was used to determine the degree of correlation between the appearance scores for TDP-43 or tau inclusions and the severity of neuronal loss, and between those for TDP-43 and tau inclusions in each anatomical region [25]. All statistical analyses were performed using SPSS Statistics version 20 (IBM, Armonk, NY, USA). Differences were considered statistically significant at $P < 0.05$.

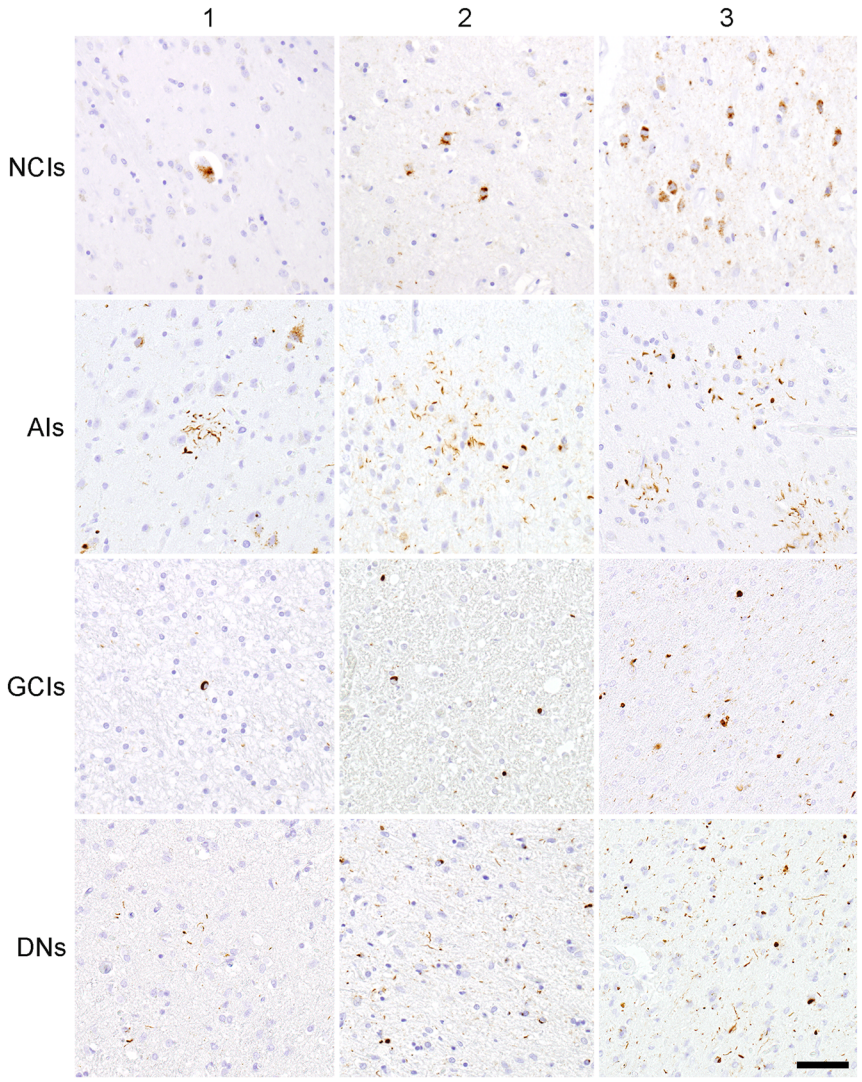

FIGURE 1 Semi-quantitative grading of TDP-43-immunolabelled inclusions according to 'appearance score'.
Representative images of grade 1 (sparse), 2 (moderate) and 3 (frequent) neuronal cytoplasmic inclusions (NCIs: putamen), astrocytic inclusions (AIs: frontal cortex), oligodendroglial cytoplasmic inclusions (GCIs: left, frontal white matter; middle, pontine tegmentum; right, cerebellar white matter) and dystrophic neurites (DNs: superior colliculus). Bar = 100 μm for all panels

RESULTS

TDP-43 inclusions

TDP-43 inclusions in the form of NCIs, AIs, GCIs or DNs were observed in various numbers in the CNS of 9 of 10 patients (Tables 1 and S1). AIs were morphologically similar to astrocytic plaques, and double-labelling immunofluorescence for phospho Ser409 TDP-43 and an astrocytic marker, ALDH1L1 [26], confirmed that TDP-43 AIs were unequivocally located in astrocytes (Figure S1).

We estimated the proportion of patients in whom each form was present in each CNS region, and the term 'Overall' was used to indicate patients in whom any forms were present in a specific region (Table 2). The incidence of 'Overall' was relatively high for the subcortical grey matter and brainstem tegmentum, and the highest score (80%) was recorded for the putamen and globus pallidus. In the majority of the regions, 'Overall' reflected the proportions of patients with neuronal inclusions (NCIs and DNs), rather than those with glial inclusions (AIs and GCIs). The proportion of patients with AIs was the same as or lower than that of patients with other forms in the majority of brain regions, except for the putamen and amygdala. The median appearance score for NCIs was high in several regions, including the putamen and globus pallidus, whereas the scores for other forms were intermediate or low in all regions (Table 2). Thus, NCIs were considered to be the most prominent form of TDP-43 inclusion.

TDP-43 inclusions and neuronal loss

We measured the degree of correlation between the quantity of TDP-43 inclusions and the severity of neuronal loss in all regions. Representative images are shown in Figure 3. The appearance score for NCIs was significantly correlated with the neuronal loss score in 11 of 20 grey matter regions, including the subcortical grey matter and midbrain (Table 3), where the median appearance score for NCIs was high or intermediate (Table 2). On the other hand, the appearance

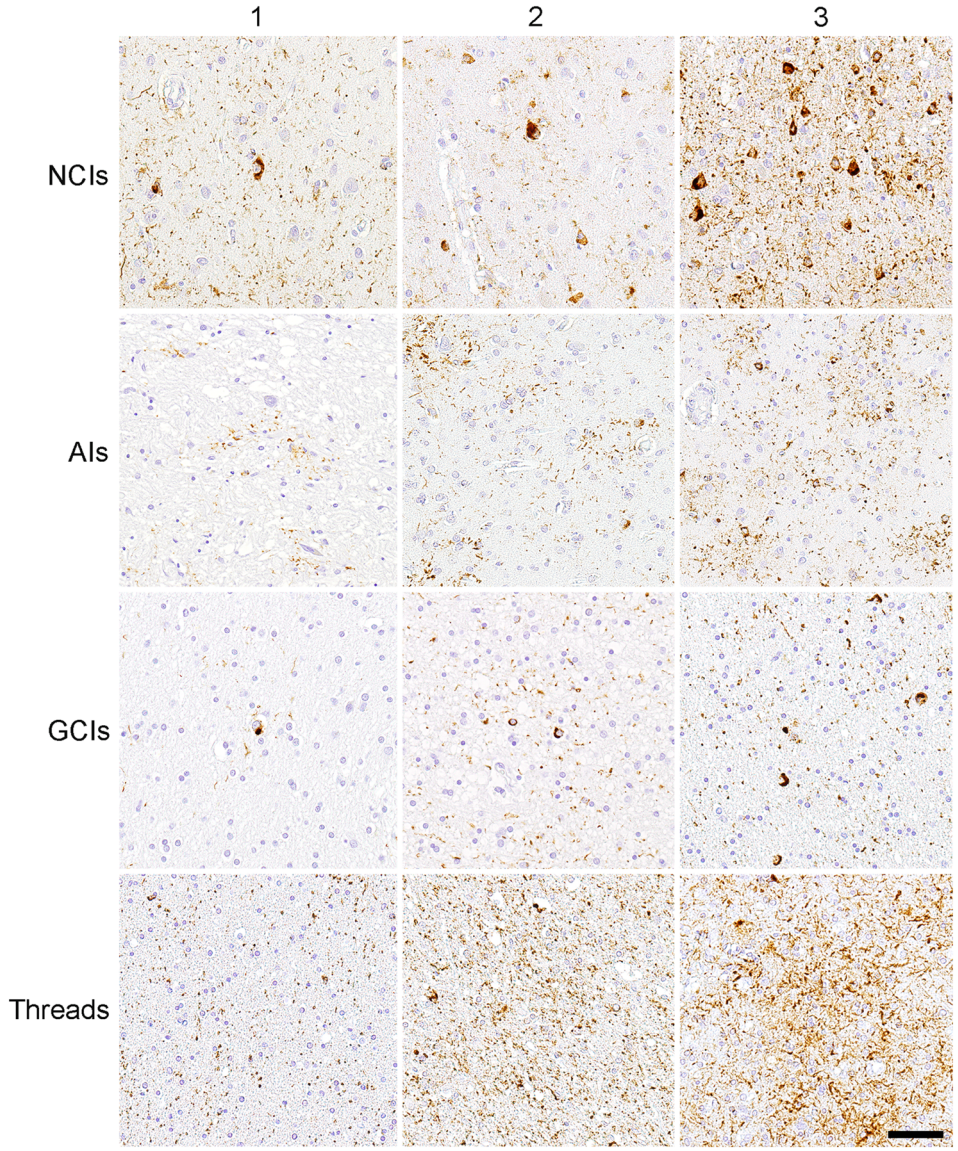

FIGURE 2 Semi-quantitative grading of tau-immunolabelled inclusions according to 'appearance score'.
Representative images of grade 1 (sparse), 2 (moderate) and 3 (frequent) neuronal cytoplasmic inclusions (NCIs: frontal cortex), astrocytic inclusions (AIs: left, pontine tegmentum; middle and right, frontal cortex), oligodendroglial cytoplasmic inclusions (GCIs: frontal white matter) and threads (frontal white matter). Bar = 100 μm for NCIs, GCIs and threads, and 130 μm for AIs

scores for AIs, GCIs and DNs were also significantly correlated with the neuronal loss score in fewer regions (4, 9 and 6 of the 22 grey and white matter regions, respectively: Table 3). In all such regions, except for the motor cortex for AIs and the medullary tegmentum for GCIs, the appearance score for NCIs was also significantly correlated with the neuronal loss score (Table 3).

Tau inclusions and neuronal loss

We also measured the degree of correlation between the quantity of tau inclusions and the severity of neuronal loss in all regions. The appearance scores for tau NCIs, AIs and threads were high in the majority of regions, except for the cerebellum and spinal cord, whereas the appearance score for GCIs was relatively low (Table S1). The appearance score for tau NCIs was significantly correlated with the neuronal loss score in 4 of 20 grey matter regions (Table S2), all of which overlapped with the regions where the appearance score for TDP-43 NCIs was significantly correlated with the neuronal loss score (Table 3). On the other hand, the appearance scores for AIs, GCIs and threads did not show a significant positive correlation with the neuronal loss score in any region except for the lumbar anterior horns for GCIs (Table S2).

TDP-43 and tau inclusions

Next, we assessed the correlation between the quantity of TDP-43 inclusions and that of tau inclusions. For tau inclusions, the appearance scores were high in the majority of regions, although the appearance score for GCIs was relatively low, as mentioned above (Table S1). In contrast to tau inclusions, the appearance scores for all forms of TDP-43 inclusions were apparently low and highly variable among the patients (Table S1). Statistical analysis of the correlation between TDP-43 and tau inclusions of each form revealed a significant positive correlation only in 4, 0 and

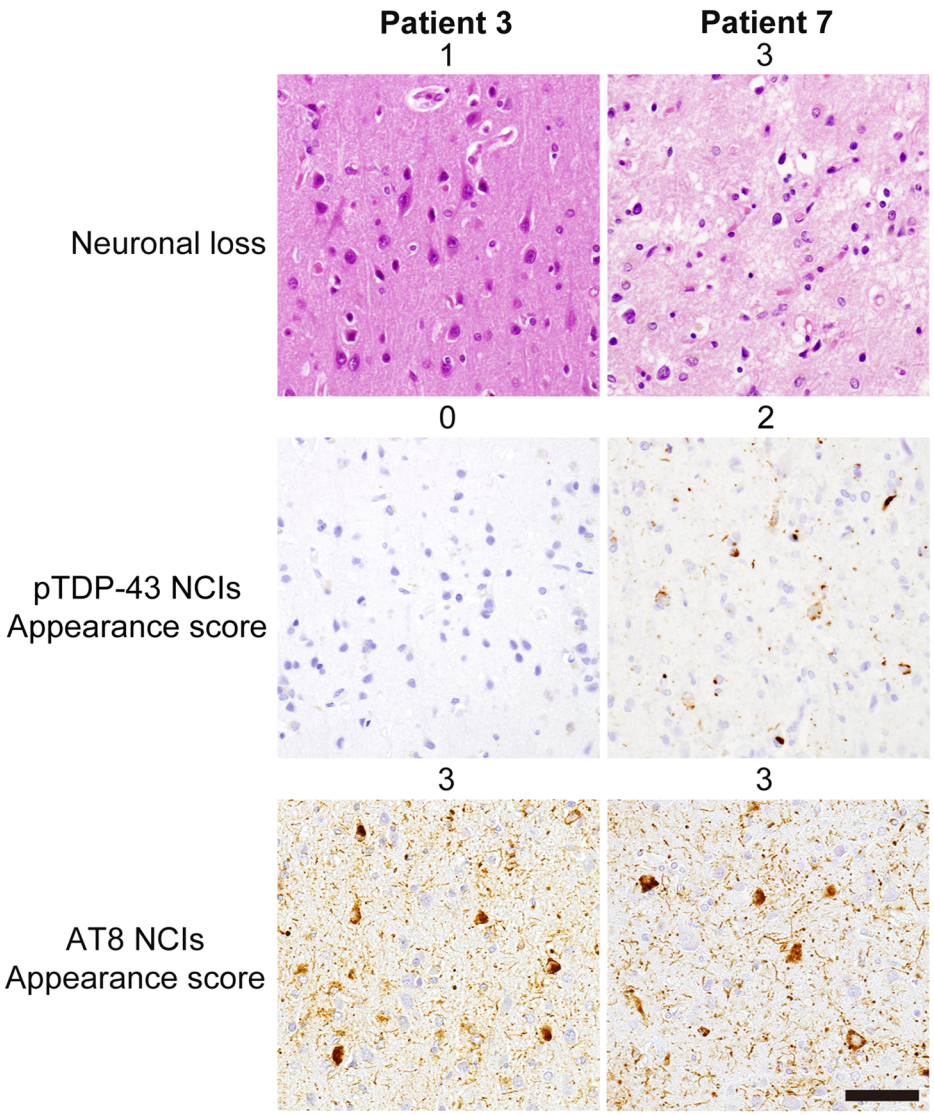

FIGURE 3 Representative images of neuronal loss, TDP-43 and tau neuronal cytoplasmic inclusions in consecutive sections.
Images taken from consecutive sections of the frontal cortex of each patient. Left, Patient 3; right, Patient 7. The severity of neuronal loss and the appearance score for TDP-43 neuronal cytoplasmic inclusions (NCIs) are higher in Patient 7 than in Patient 3, while the appearance scores for tau NCIs are the same. Bar = 100 μm for all panels

4 regions for NCIs, AIs and GCIs, respectively (Table 4). Even in the four regions where both the appearance scores for TDP-43 NCIs and tau NCIs were significantly correlated with the neuronal loss score, the correlation between TDP-43 and tau NCIs was significant only in the dentate nucleus. Thus, the appearance of TDP-43 was not correlated with that of tau in the majority of regions.

Double-labelling immunofluorescence for TDP-43 and tau

Double-labelling immunofluorescence staining revealed that TDP-43 NCIs, DNs and GCIs were present in tau-positive and tau-negative cytoplasm and processes (Figure 4A–C). On the other hand, TDP-43 AIs were frequently colocalized with tau AIs, although the processes of tau AIs were more numerous than those of TDP-43 AIs (Figure 4D–F). Thus, the modes of aggregation of TDP-43 and tau may differ among the cell types.

DISCUSSION

In the present study, we demonstrated that the appearance of TDP-43 NCIs was significantly correlated with neuronal loss in CBD (Table 3). Moreover, we found no close correlation between the burden of TDP-43 and that of tau in neurons (Table 4). We also found that the regions in which TDP-43 AIs and GCIs were significantly related to neuronal loss were fewer than was the case for TDP-43 NCIs (Table 3), indicating that the impact of glial inclusions may be lower than that of neuronal inclusions.

Considering the possibility that FTLD-TDP [27] or LATE [28] might have co-occurred with CBD in the present patients, since TDP-43 inclusions were observed most frequently in the putamen and globus pallidus where NCIs showed the highest appearance score, and no patients showed restriction or accentuation of TDP-43 inclusion distribution in the frontal lobe or medial temporal lobe, the distribution pattern of TDP-43 inclusions was distinct from that usually seen in these diseases or in an unclassified limbic-predominant TDP-43 proteinopathy previously reported to contribute to the pathology in

TABLE 3 Regional correlations between the severity of neuronal loss and the quantity of TDP-43 inclusions

	NCIs		AIs		GCIs		DNs	
CNS regions	Gamma	*P*	Gamma	*P*	Gamma	*P*	Gamma	*P*
Frontal cortex	1.00	<0.01	0.91	<0.01	1.00	<0.01	1.00	<0.01
Frontal white matter*	na	na	0.20	0.77	0.20	0.77	0.27	0.65
Motor cortex	0.77	0.05	1.00	0.02	0.60	0.13	0.46	0.29
Putamen	0.92	<0.01	0.65	0.08	0.88	0.02	1.00	<0.01
Globus pallidus	0.74	0.02	0.75	0.05	0.33	0.55	1.00	0.05
Thalamus	0.52	0.03	0.90	<0.01	0.86	<0.01	0.69	<0.01
Subthalamic nucleus	0.75	<0.01	0.29	0.55	0.83	0.01	0.83	0.01
Amygdala	0.77	<0.01	0.85	<0.01	1.00	<0.01	0.60	0.05
Ammon CA1	0.46	0.18	0.67	0.12	0.24	0.53	0.44	0.22
Dentate gyrus	0.66	0.06	-	-	0.57	0.18	0.60	0.10
Midbrain tegmentum	0.82	0.01	0.50	0.16	0.82	0.01	0.25	0.51
Superior colliculus	0.67	0.01	0.40	0.30	0.57	0.09	0.39	0.18
Substantia nigra	1.00	<0.01	0.67	0.26	1.00	<0.01	0.80	0.01
Pontine tegmentum	0.20	0.69	-0.11	0.87	0.47	0.31	0.47	0.31
Pontine nuclei	-0.46	0.48	-1.00	0.30	-0.07	0.92	0.20	0.77
Medullary tegmentum	0.43	0.46	-	-	1.00	0.03	0.67	0.13
Inferior olive	1.00	0.04	0.25	0.75	0.85	0.10	1.00	0.09
Cerebellar cortex	-	-	-	-	0.83	0.11	0.33	0.49
Cerebellar white matter*	na	na	-	-	0.14	0.80	0.43	0.42
Dentate nucleus	1.00	<0.01	-	-	1.00	<0.01	1.00	<0.01
Anterior horns (C)	0.33	0.60	-	-	-0.60	0.34	0.33	0.59
Anterior horns (L)	-1.00	0.29	-	-	-1.00	0.29	0.43	0.60

Note: Gamma, Goodman-Kruskal Gamma index: −1 (negative correlation) ≦ G ≦1 (positive correlation). —, because the appearance scores for each form of TDP-43 inclusion in these regions were identical in all patients, Goodman-Kruskal Gamma index was not applicable.

, Regions where the appearance scores for each form of TDP-43 inclusion were correlated significantly with the neuronal loss scores.

Abbreviations: AIs, astrocytic inclusions; C, cervical; DNs, dystrophic neurites; GCIs, glial cytoplasmic inclusions; L, lumbar; na, not applicable; NCIs, neuronal cytoplasmic inclusions.

*Correlation between the severity of loss of myelinated fibres and the quantity of TDP-43 inclusions.

CBD patients [29]. Furthermore, the appearance score for AIs was equal to, or higher than that of, NCIs in the frontal cortex in all five patients who had TDP-43 inclusions in this region (Table S1). Such cortical features with prominent AIs could not have corresponded to those of any FTLD-TDP subtypes [27]. Thus, it seemed unlikely that FTLD-TDP or LATE had co-occurred in the present patients.

The impact of TDP-43 accumulation on the cellular pathomechanisms underlying neuronal degeneration and death has been largely uncertain. The present findings suggest that TDP-43 protein aberration may indeed be associated with neuronal degeneration in CBD. Consistent with this, several previous studies have demonstrated a higher frequency of TDP-43 pathology in patients with CBD than in those with other tauopathies, including PSP [3, 6–9] and Pick disease [3, 6, 8]. Moreover, biochemical studies have demonstrated that misfolded tau and TDP-43 interact to exert deleterious effects such as neuronal cell death [30], supporting a possible role for TDP-43 in the pathomechanism of tauopathy. However, in patients with CBD—also a representative four-repeat tauopathy—our semi-quantitative and topographical study demonstrated no clear

TABLE 4 Regional correlations between the quantities of TDP-43 and tau inclusions

CNS regions	NCIs		AIs		GCIs	
	Gamma	*P*	Gamma	*P*	Gamma	*P*
Frontal cortex	0.53	0.25	1.00	0.26	0.33	0.55
Frontal white matter	na	na	0.57	0.24	0.57	0.24
Motor cortex	1.00	0.26	-	-	-0.17	0.55
Putamen	1.00	<0.01	-	-	-1.00	<0.01
Globus pallidus	0.50	0.30	-0.43	0.44	0.77	0.07
Thalamus	0.16	0.68	-0.67	0.26	-0.70	0.06
Subthalamic nucleus	0.80	0.07	-0.25	0.74	0.08	0.88
Amygdala	0.46	0.43	1.00	0.25	-1.00	0.07
Ammon CA1	1.00	0.05	1.00	0.13	0.71	0.11
Dentate gyrus	1.00	<0.01	-	-	1.00	0.28
Midbrain tegmentum	1.00	0.25	1.00	0.25	0.70	0.05
Superior colliculus	0.50	0.16	1.00	0.26	1.00	<0.01
Substantia nigra	-0.14	0.71	-	-	-0.63	0.28
Pontine tegmentum	0.46	0.38	1.00	0.13	0.73	0.01
Pontine nuclei	-0.65	0.13	1.00	0.25	-0.11	0.87
Medullary tegmentum	-0.08	0.88	-	-	0.11	0.86
Inferior olive	0.81	0.02	1.00	0.28	-0.33	0.42
Cerebellar cortex	-	-	-	-	0.33	0.65
Cerebellar white matter	na	na	-	-	0.80	0.04
Dentate nucleus	1.00	<0.01	-	-	0.73	0.04
Anterior horns (C)	1.00	0.26	-	-	-1.00	0.30
Anterior horns (L)	1.00	0.25	-	-	1.00	0.29

Note: Gamma, Goodman-Kruskal Gamma index: −1 (negative correlation) ≦ G ≦1 (positive correlation). −, because the appearance scores for each form of TDP-43 inclusion or tau inclusion in these regions were identical in all patients, Goodman-Kruskal Gamma index was not applicable.
, Regions showing significantly positive or negative correlations between the appearance scores for each form of TDP-43 inclusion and that of tau inclusion, respectively.
Abbreviations: AIs, astrocytic inclusions; C, cervical; DNs, dystrophic neurites; GCIs, glial cytoplasmic inclusions; L, lumbar; na, not applicable; NCIs, neuronal cytoplasmic inclusions.

correlation between TDP-43 NCIs and tau NCIs (Table 4), and our double-immunolabelling study revealed that TDP-43 accumulated frequently in tau-negative neurons (Figure 4), implying no direct involvement of both proteins in vivo.

In comparison with two previous studies of largely Caucasian patients with CBD in whom the brain burden of TDP-43 inclusions was 33% [9] and 45% [7], the proportion of affected patients in the present study was as high as 90% (Tables 1 and S1). This disparity may be due partly to the difference in genetic background between the patient populations. For example, it has been shown that the H1 haplotype of the *MAPT* gene [31, 32] and *C9orf72* intermediate repeats (≥17 repeats) [33] are associated with the occurrence of CBD in Caucasian populations. However, in the Japanese population, only the H1/H1 haplotype has been identified (Table S3) [34] and repeat expansion in the *C9orf72* gene is quite rare [35–37]. Genetic analysis of eight of the present patients for whom sufficient tissue samples were available confirmed that there were no missense mutations in any of the coding regions, copy number alterations of the *MAPT* gene or any repeat expansions (up to seven repeats) in the *C9orf72* gene. In addition, the *MAPT* haplotype was H1/H1, as expected, and the *APOE*

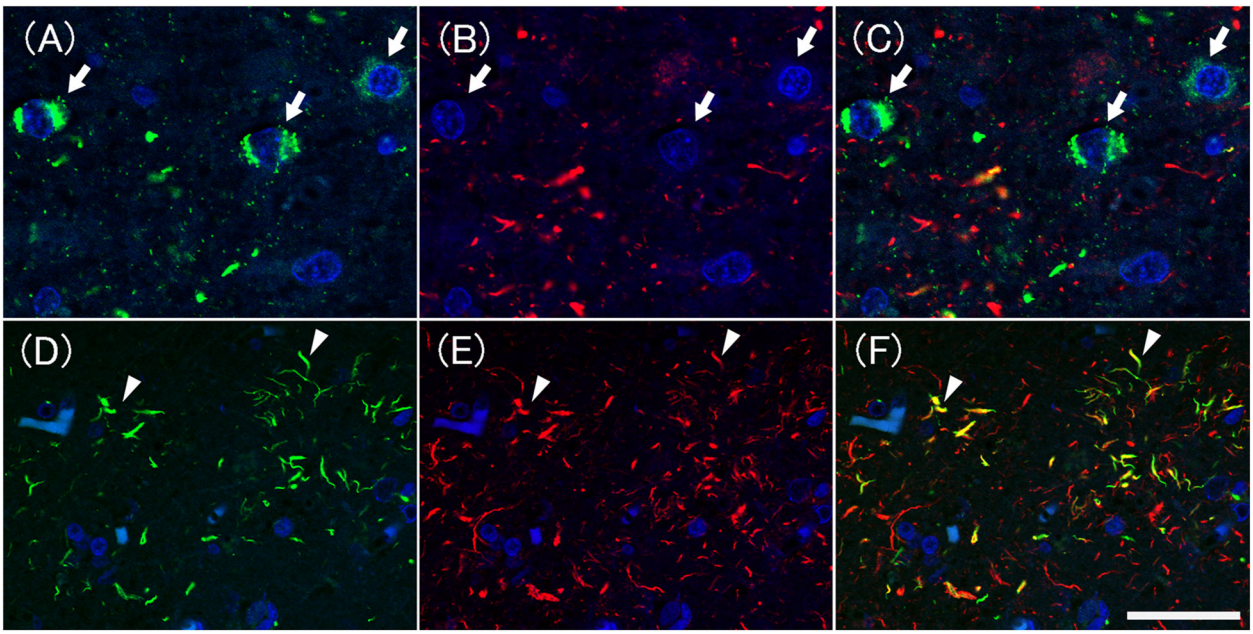

FIGURE 4 Double immunofluorescence images for TDP-43 and tau.
(A–C) Phosphorylated TDP-43 (pTDP-43)-positive and tau-negative neuronal cytoplasmic inclusions (arrows) and tau-positive and pTDP-43-negative or partially positive threads in the putamen (Patient 3). (D–F) Phosphorylated TDP-43 and tau are partially co-localised in astrocytic plaques (arrowheads) in the motor cortex (Patient 1). Green: anti-phospho Ser409 TDP-43, red: AT8. Bar = 40 μm for all panels

genotype was 3*3 in all patients (Table S3). With regard to *GRN* and *TMEM106B*, which have been reported to be risk modulator genes for FTLD-TDP [38, 39], it is interesting to note that one of the eight patients was homozygous for the minor allele of *GRN* rs5848, which has been shown to increase the risk of FTLD-TDP [38], and that the each of the other seven patients had one of the minor alleles, whereas the frequency of the minor allele of *TMEM106B* rs3173615 was similar to that in the Japanese population as a whole (Table S3). A previous genetic analysis of Caucasian populations has shown that the frequency of the H1/H1 haplotype in TDP-43-severe CBD is low [7]. Therefore, further studies will be necessary to clarify the correlation between genetic background and the TDP-43 burden in patients with CBD.

A large cohort study of patients with CBD demonstrated an association between severe TDP-43 pathology and the PSPS clinical phenotype, and the TDP-43 pathology being most severe and frequent in the midbrain tegmentum and subthalamic nucleus, which are preferentially affected in PSP [7]. Consistent with this, the present study showed that TDP-43 NCIs were observed frequently in both regions (Table 2) and quantitatively correlated with neuronal loss (Table 3). However, we failed to demonstrate any significant impact of TDP-43 pathology and neuronal loss on the clinical phenotype (data not shown), perhaps reflecting the small number of autopsied patients that were recruited.

Among other neurodegenerative factors observed in the present patients, seven had argyrophilic grains, and two had Lewy bodies (Table 1). Considerable variation in the frequency of argyrophilic grains in patients with CBD has been reported, ranging from 41.2% to 100% [40]. Although the frequency of 70% in the present study was lower than that in a previous study of CBD in the Japanese population [41], methodological differences might have been responsible. There were no significant differences in neuronal loss score, or in the appearance score for any form of TDP-43 inclusion, between patients with and without argyrophilic grains in amygdala ($P = 0.15$ for neuronal loss, 0.81 for TDP-43 NCIs and 0.60 for TDP-43 DNs). Therefore, in our study sample, argyrophilic grains did not appear to affect the severity of TDP-43 pathology or neuronal loss. On the other hand, in Patient 2, several α-synuclein inclusions were observed only in the amygdala, whereas Patient 4 had several in the medullary tegmentum and a few in the substantia nigra and amygdala. Considering this restricted distribution and small number of α-synuclein inclusions, it appeared that they did not contribute significantly to neuronal loss in these patients.

In conclusion, the present study has demonstrated a significant topographical correlation between neuronal cytoplasmic aggregation of TDP-43 and neuronal loss, suggesting that TDP-43 protein aberration might be associated with neuronal degeneration in CBD.

ACKNOWLEDGEMENTS

This work was supported in part by JSPS Grants-in-aid for Scientific Research to MT (19K07972), and AK (19H01061 and 19H05559), Grants-in-Aid, Japan Agency for Medical Research and Development (JP21dk0207045 and JP21ek0109545) to TI, and Grants-in Aid from the Research Committee of CNS Degenerative Diseases, Research on Policy Planning and Evaluation for Rare and Intractable Diseases, Health, Labor and Welfare Sciences Research Grants, the Ministry of Health, Labour and Welfare, Japan to AK.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

ETHICS STATEMENT

This study was approved by the Institutional Review Board of Niigata University School of Medicine, Niigata, Japan (G2019-0020).

AUTHOR CONTRIBUTIONS

MS, MT and AK designed the research project, performed the pathological analysis and were responsible for writing the manuscript. YF, NH, AM and TI performed the genetic analysis. KT, JI, IA, TN and OO examined the patients and conducted the clinical analysis. KA performed the statistical analyses. TI, OO and AK managed the study.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/nan.12786.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Sainouchi M, Tada M, Fitrah YA, et al. Brain TDP-43 pathology in corticobasal degeneration: Topographical correlation with neuronal loss. *Neuropathol Appl Neurobiol*. 2022;e12786. doi:10.1111/nan.12786