

context of present findings, if the degree of  $\alpha$ -synuclein pathology in this series [20] was mild, the effect of  $\alpha$ -synuclein pathology on the development of TDP-43 accumulation would not likely be demonstrated.

Although multivariate analysis in our study failed to demonstrate a significant association between tau pathology and TDP-43 accumulation, the result does not necessarily deny the possible effect of tau pathology in LBD-Ltau cases. Since our study was conducted to mainly examine the effect of  $\alpha$ -synuclein pathology, the proportion of subjects having severe tau pathology of Braak NFT stages V–VI was low, less than 10%. The low proportion of this subgroup might therefore have influenced our results. Nevertheless, it is notable that tau burden in the hippocampal dentate gyrus tended to be more severe in TDP-43-positive LBD cases, and that tau was often colocalized with TDP-43 in the amygdala. The independent effects on the development of TDP-43 accumulation of tau pathology, as well as that of  $\alpha$ -synuclein pathology, need to be further examined in future studies using a multivariate model in a larger number of cases with various degrees of tau and  $\alpha$ -synuclein pathologies.

The pathophysiological mechanism underlying the coexistence of  $\alpha$ -synuclein and TDP-43 accumulations in the same LBD case remains unclear. It has been reported that some FTLTDP cases with progranulin gene mutations had concomitant  $\alpha$ -synuclein pathology [5, 24], although the frequency is not high: in a previous study, only one of 18 cases (5.5%) of FTLTDP with ubiquitin-positive inclusion (FTLTDP-U) had Lewy pathology [18]. It was also reported that one case of familial PD ( $\alpha$ -synuclein A53T heterozygote) had TDP-43 pathology [26]. To our knowledge, the frequency of TDP-43 pathology in familial LBD cases has not been examined. Colocalization of TDP-43 and  $\alpha$ -synuclein in DLB cases was demonstrated in two studies [2, 14], being consistent with our findings. An ultrastructural study also demonstrated that filaments and granular material associated with  $\alpha$ -synuclein filaments in Lewy bodies were labeled with anti-TDP-43 antibodies [25].

It is difficult to draw any definite conclusions regarding the biological mechanism underlying the coexistence of  $\alpha$ -synuclein and TDP-43 in the same neuron or the same case. However, the results presented in this paper suggest that the limbic system, and in particular the amygdala, is vulnerable to the deposition of TDP-43 in LBD, as well as other degenerative diseases including AD [1, 2, 15], AGD [8], and PSP [34]. Therefore, TDP-43 deposition in the amygdala may be a region-specific rather than disease-specific phenomenon. In LBD,  $\alpha$ -synuclein deposition in the limbic region may be primary, and TDP-43 secondarily deposits upon pre-existing LBs, generating some colocalization. This hypothesis seems to be supported by

observations that some TDP-43-positive inclusions in our cases showed typical morphological features of LBs. However, the existence of TDP-43 deposited separately from  $\alpha$ -synuclein accumulation may also suggest that TDP-43 accumulation cannot only be explained by some direct biological synergy between the proteins. Further, severe neurodegeneration associated with  $\alpha$ -synuclein deposition might indirectly lead TDP-43 to accumulate in the vulnerable regions, in particular the amygdala. In addition, the possibility that the accumulation of TDP-43 might be associated with aging should not be excluded. Indeed, some previous studies have demonstrated that aging influences the accumulation of TDP-43 in AD [2, 20] and PDD [28], with age at death being later in cases with TDP-43 changes than in those without. Recently, Geser et al. [10] proposed that TDP-43 proteinopathies could be divided into two categories, major TDP-43 proteinopathies (e.g., ALS and FTLTDP [3, 6, 7, 29]) and disorders with secondary TDP-43 pathologies (e.g., AD [1, 2, 15], LBD [2, 14, 28], AGD [8], CBD [32], and PSP [34]). Potential mechanisms regarding TDP-43 accumulation (as mentioned above) could be associated with the pathophysiology in the latter category.

TDP-43 pathology is known to be strongly associated with the development of hippocampal sclerosis. For example, it was reported that 59 of 75 cases (79%) of FTLTDP with ubiquitin-positive inclusions (FTLTDP-U) had hippocampal sclerosis [19], 8 of 11 cases (73%) of pure hippocampal sclerosis had TDP-43-positive inclusions [1]. With respect to neuronal loss in the hippocampus in LBD cases with TDP-43 pathology, Nakashima-Yasuda et al. [28] reported that the frequency of hippocampal sclerosis was 60% of 25 TDP-43-positive DLB + AD cases, and 50% of 4 TDP-43-positive PDD cases, and none of 5 TDP-43-positive PD cases. Why none of the present LBD cases with TDP-43 pathology had hippocampal sclerosis is unclear. However, one plausible explanation is that TDP-43 pathology in our LBD series might be less severe than that in LBD series by Nakashima-Yasuda: all of their TDP-43-positive LBD cases had the labeled inclusions in the hippocampal dentate gyrus, while only 30% of TDP-43-positive cases in our series had the lesions in the site. As with the relationship with hippocampal sclerosis, it is also plausible that the severity of TDP-43 pathology might have an impact on clinical presentation in LBD cases. However, the fact that no clear association between TDP-43 pathology and presence or absence of dementia was noted in our LBD cases might be explained by the relatively mild TDP-43 pathology in our series. The relationship between the severity of TDP-43 pathology and the development of hippocampal sclerosis, and the effects of these pathological parameters on clinical presentation in LBD cases should be examined in the future.

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## References

- Amador-Ortiz C, Lin WL, Ahmed Z et al (2007) TDP-43 immunoreactivity in hippocampal sclerosis and Alzheimer's disease. *Ann Neurol* 61:435–445
- Arai T, Mackenzie IR, Hasegawa M et al (2009) Phosphorylated TDP-43 in Alzheimer's disease and dementia with Lewy bodies. *Acta Neuropathol* 117:125–136
- Arai T, Hasegawa M, Akiyama H et al (2006) TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochem Biophys Res Commun* 351:602–611
- Braak H, Alafuzoff I, Arzberger T, Kretschmar H, Del Tredici K (2006) Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. *Acta Neuropathol* 112:389–404
- Cairns NJ, Neumann M, Bigio EH et al (2007) TDP-43 in familial and sporadic frontotemporal lobar degeneration with ubiquitin inclusions. *Am J Pathol* 171:227–240
- Cairns NJ, Bigio EH, Mackenzie IR et al (2007) Neuropathologic diagnostic and nosologic criteria for frontotemporal lobar degeneration: consensus of the Consortium for Frontotemporal Lobar Degeneration. *Acta Neuropathol* 114:5–22
- Davidson Y, Kelley T, Mackenzie IR et al (2007) Ubiquitinated pathological lesions in frontotemporal lobar degeneration contain the TAR DNA-binding protein, TDP-43. *Acta Neuropathol* 113:521–533
- Fujishiro H, Uchikado H, Arai T et al (2009) Accumulation of phosphorylated TDP-43 in brains of patients with argyrophilic grain disease. *Acta Neuropathol* 117:151–158
- Geser F, Winton MJ, Kwong LK et al (2008) Pathological TDP-43 in parkinsonism-dementia complex and amyotrophic lateral sclerosis of Guam. *Acta Neuropathol* 115:133–145
- Geser F, Martinez-Lage M, Kwong LK et al (2009) Amyotrophic lateral sclerosis, frontotemporal dementia and beyond: the TDP-43 diseases. *J Neurol* 256:1205–1214
- Hansen L, Salmon D, Galasko D et al (1990) The Lewy body variant of Alzheimer's disease: a clinical and pathologic entity. *Neurology* 40:1–8
- Hasegawa M, Arai T, Akiyama H et al (2007) TDP-43 is deposited in the Guam parkinsonism-dementia complex brains. *Brain* 130:1386–1394
- Hasegawa M, Arai T, Nonaka T et al (2008) Phosphorylated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Ann Neurol* 64:60–70
- Higashi S, Iseki E, Yamamoto R et al (2007) Concurrence of TDP-43, tau and alpha-synuclein pathology in brains of Alzheimer's disease and dementia with Lewy bodies. *Brain Res* 1184:284–294
- Hu WT, Josephs KA, Knopman DS et al (2008) Temporal lobar predominance of TDP-43 neuronal cytoplasmic inclusions in Alzheimer disease. *Acta Neuropathol* 116:215–220
- Inukai Y, Nonaka T, Arai T et al (2008) Abnormal phosphorylation of Ser409/410 of TDP-43 in FTLD-U and ALS. *FEBS Lett* 582:2899–2904
- Jellinger KA (2010) The neuropathologic substrate of Parkinson disease dementia. *Acta Neuropathol* 119:151–153
- Josephs KA, Ahmed Z, Katsuse O et al (2007) Neuropathologic features of frontotemporal lobar degeneration with ubiquitin-positive inclusions with progranulin gene (PGRN) mutations. *J Neuropathol Exp Neurol* 66:142–151
- Josephs KA, Dickson DW (2007) Hippocampal sclerosis in tau-negative frontotemporal lobar degeneration. *Neurobiol Aging* 28:1718–1722
- Josephs KA, Whitwell JL, Knopman DS et al (2008) Abnormal TDP-43 immunoreactivity in AD modifies clinicopathologic and radiologic phenotype. *Neurology* 70:1850–1857
- Kalaitzakis ME, Graeber MB, Gentleman SM, Pearce RK (2008) Striatal beta-amyloid deposition in Parkinson disease with dementia. *J Neuropathol Exp Neurol* 67:155–161
- Kalaitzakis ME, Pearce RK (2009) The morbid anatomy of dementia in Parkinson's disease. *Acta Neuropathol* 118:587–598
- Kosaka K (1990) Diffuse Lewy body disease in Japan. *J Neurol* 237:197–204
- Leverenz JB, Yu CE, Montine TJ (2007) A novel progranulin mutation associated with variable clinical presentation and tau, TDP43 and alpha-synuclein pathology. *Brain* 130:1360–1374
- Lin WL, Dickson DW (2008) Ultrastructural localization of TDP-43 in filamentous neuronal inclusions in various neurodegenerative diseases. *Acta Neuropathol* 116:205–213
- Markopoulou K, Dickson DW, McComb RD (2008) Clinical, neuropathological and genotypic variability in SNCA A53T familial Parkinson's disease. Variability in familial Parkinson's disease. *Acta Neuropathol* 116:25–35
- McKeith IG, Dickson DW, Lowe J et al (2005) Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. *Neurology* 65:1863–1872
- Nakashima-Yasuda H, Uryu K et al (2007) Co-morbidity of TDP-43 proteinopathy in Lewy body related diseases. *Acta Neuropathol* 114:221–229
- Neumann M, Sampathu DM, Kwong LK et al (2006) Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* 314:130–133
- Obi K, Akiyama H, Kondo H et al (2008) Relationship of phosphorylated alpha-synuclein and tau accumulation to Abeta deposition in the cerebral cortex of dementia with Lewy bodies. *Exp Neurol* 210:409–420
- The National Institute on Aging, and Reagan Institute Working Group (1997) Consensus recommendations for the postmortem diagnosis of Alzheimer disease. The National Institute on Aging, and Reagan Institute Working Group on diagnostic criteria for the neuropathological assessment of Alzheimer disease. *Neurobiol Aging* 18:S1–S2
- Uryu K, Nakashima-Yasuda H, Forman MS et al (2008) Concomitant TAR-DNA-binding protein 43 pathology is present in Alzheimer disease and corticobasal degeneration but not in other tauopathies. *J Neuropathol Exp Neurol* 67:555–564
- Yokota O, Tsuchiya K, Uchihara T et al (2007) Lewy body variant of Alzheimer's disease or cerebral type Lewy body disease? Two autopsy cases of presenile onset with minimal involvement of the brainstem. *Neuropathology* 27:21–35
- Yokota O, Davidson Y, Bigio EH et al (2010) Phosphorylated TDP-43 pathology and hippocampal sclerosis in progressive supranuclear palsy. *Acta Neuropathol* 120:55–66

## Phosphorylated TDP-43 pathology and hippocampal sclerosis in progressive supranuclear palsy

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**Abstract** TDP-43 is characteristically accumulated in TDP-43 proteinopathies such as frontotemporal lobar degeneration and motor neurone disease, but is also present in some tauopathies, including Alzheimer's disease, argyrophilic grain disease, and corticobasal degeneration (CBD). However, several studies have suggested that cases of progressive supranuclear palsy (PSP) lack TDP-43 pathology. We have therefore examined limbic regions of the brain in 19 PSP cases, as well as in 12 CBD cases, using phosphorylation-dependent anti-TDP-43 antibodies. We observed TDP-43-positive inclusions in five PSP cases (26%), as well as in two CBD cases (17%). The amygdala and hippocampal dentate gyrus were most frequently affected in PSP. Regional tau burden tended to be higher in TDP-43-positive PSP cases, and a significant correlation between tau and TDP-43 burden was noted in the

occipitotemporal gyrus. Hippocampal sclerosis (HS) was found in 3/5 TDP-43-positive PSP cases, but HS was significantly more frequent in TDP-43-positive than TDP-43 negative PSP cases. Dementia was present in 13/19 (58%) of the PSP cases, in 4/5 TDP-43-positive cases, in all 3 TDP-43-positive cases with HS, in 1/2 TDP-43-positive cases without HS, and 7/14 cases lacking both. TDP-43 and tau were frequently colocalized in the amygdala, but not in the hippocampal dentate gyrus. Immunoblotting demonstrated the characteristic (for TDP-43 proteinopathies) 45 and 25 kDa bands and high molecular weight smear in the TDP-43-positive PSP case. These findings suggest that (1) although PSP is nominally a tauopathy, pathological TDP-43 can accumulate in the limbic system in some cases, and (2) TDP-43 pathology may be concurrent with HS.

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## Introduction

Transactivation-responsive DNA-binding protein of M<sub>r</sub> 43 kDa (TDP-43) is a nuclear protein involved in transcriptional repression and alternative splicing. It was originally identified as a major component of ubiquitin-positive and tau-negative inclusions in the frontotemporal cortex and motor neurons in frontotemporal lobar degeneration (FTLD-U), with or without progranulin gene mutations, and in amyotrophic lateral sclerosis (ALS) [3, 12, 31]. Subsequent studies revealed that TDP-43 is also abnormally accumulated in familial FTLD-U with mutations in the valosin-containing protein gene [32], in familial FTLD with motor neuron disease linked to chromosome 9p [10], and in ALS with TDP-43 gene mutations [25, 38, 41, 44]. TDP-43 is considered to play an essential pathogenic role in these diseases, now-called TDP-43 proteinopathies.

Although TDP-43 accumulation was originally considered to be a specific disease marker for FTLD-U and ALS, subsequent studies demonstrated that abnormal TDP-43 accumulation in some cases of other neurodegenerative diseases, such as Alzheimer's disease (AD) [2], Parkinson's disease with and without dementia [30], dementia with Lewy bodies (DLB) + AD [4, 30], ALS/parkinson-dementia complex of Guam (ALS/PDC of Guam) [15, 16], argyrophilic grain disease (AGD) [14], and Huntington disease [37]. However, the pathophysiological significance of concurrent TDP-43 accumulation, and its impact on clinical phenotype in these diseases remain unclear.

Several previous studies have suggested that cases of progressive supranuclear palsy (PSP) lack abnormal TDP-43 accumulation [3, 18, 40]. In these early studies, phosphorylation-independent antibodies were employed in TDP-43 immunohistochemistry and immunoblot analysis. We have made polyclonal and monoclonal antibodies specific for phosphorylated TDP-43, which identify phosphorylation sites in the C-terminus of the TDP-43 accumulated in FTLD-TDP brains [17, 20], and selectively immunolabel pathological inclusions and dystrophic neurites without physiological nuclear staining in FTLD-TDP, ALS, AD with TDP-43 pathology, and in DLB with TDP-43 [4, 17]. They also recognize hyperphosphorylated TDP-43 at 45 kDa and additional 18–26 kDa fragments in sarkosyl-insoluble fractions on immunoblotting.

The principal aim of this study was to revisit the presence or absence, and the frequency, of TDP-43 pathology in PSP cases using a phosphorylation-dependent

anti-TDP-43 antibody. In contrast to previous reports, we demonstrated that a significant proportion of PSP cases had variable degrees of TDP-43 pathology in the limbic system. We subsequently examined the relationships between TDP-43 pathology, tau pathology, and hippocampal sclerosis, as well as biochemical nature of the abnormally accumulated TDP-43, in PSP.

## Materials and methods

### Subjects

We investigated 19 pathologically confirmed PSP cases, 12 pathologically confirmed corticobasal degeneration (CBD) cases and 4 pathologically normal control subjects (Table 1). These cases were obtained from UK Parkinson's Disease Society Tissue Bank (7 PSP and 4 control cases), Department of Pathology, Northwestern University Feinberg School of Medicine Cognitive Neurology and Alzheimer Disease Center (5 PSP and 7 CBD cases), and Department of Neuropsychiatry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences (7 PSP and 5 CBD cases). All brains had been collected with Local Research Ethical Committee approval. All PSP cases showed characteristic tufted astrocytes, and all CBD cases astrocytic plaques, as revealed by Gallyas-Braak silver methods and tau immunohistochemistry.

### Immunohistochemistry

Sections cut at 5- $\mu$ m thickness to include the amygdala, entorhinal cortex, hippocampus, occipitotemporal cortex in all cases, as well as the substantia nigra in two cases for which tissue was available, were stained with antibodies against phosphorylated TDP-43 (pAb pS409/410, rabbit, polyclonal, 1:1,000 [17]), phosphorylated tau (AT8, mouse, monoclonal, 1:3,000, Innogenetics, Ghent, Belgium), phosphorylated  $\alpha$ -synuclein (#1175, rabbit, polyclonal, 1:1,000, [33]), and A $\beta$  (4G8, mouse, monoclonal, 1:2,000, Covance Research Products Inc., Dedham, MA, USA). Deparaffinized sections were incubated with 1% H<sub>2</sub>O<sub>2</sub> in methanol for 20 min to eliminate endogenous peroxidase activity in the tissue. When using anti- $\alpha$ -synuclein and anti-TDP-43 antibodies, sections were pretreated to enhance immunoreactivity in a microwave oven for 5 min in 10 mM sodium citrate buffer, pH 6.0, at 100°C. After blocking with 10% normal serum, sections were incubated 1 h at room temperature with the primary antibody. After three 5-min washes in phosphate-buffered saline (PBS), sections were incubated in biotinylated secondary antibody for 30 min, and then in avidin-biotinylated horseradish peroxidase complex (ABC Elite kit, Vector, Burlingame, CA, USA) for



**Table 1** Demographic data in PSP and CBD cases with and without TDP-43 pathologies

	PSP			CBD		
	All	TDP-43-positive PSP	TDP-43-negative PSP	All	TDP-43-positive CBD	TDP-43-negative CBD
<i>N</i> (%)	19	5 (26.3)	14 (73.7)	12	2 (16.7)	10 (83.3)
Male [ <i>N</i> (%)]	16 (84.2)	4 (80.0)	12 (85.7)	7 (58.3)	1 (50.0)	6 (60.0)
Age at onset [mean (SD)]	68.3 (9.8)	75.0 (9.4)	65.7 (9.0)	55.2 (10.2)	49.0 (12.7)	56.6 (9.9)
Age at death [mean (SD)]	76.3 (10.7)	82.4 (11.7)	74.1 (9.8)	62.8 (11.2)	56.0 (15.6)	64.1 (10.7)
Duration [mean (SD)]	7.4 (4.4)	7.4 (4.6)	7.5 (4.6)	7.3 (2.9)	7.0 (2.8)	7.3 (3.1)
Dementia (%)	11 (57.9)	4 (80.0)	7 (50.0)	11 (91.7)	2 (100.0)	9 (90.0)
Brain weight [g, mean (SD)]	1,202 (142)	1,234 (180)	1,190 (132)	1,174 (146)	1,008 (152)	1,215 (120)
Argyrophilic grains [ <i>N</i> (%)]	4 (21.1)	1 (20.0)	3 (21.4)	3 (25.0)	1 (50.0)	2 (20.0)
Hippocampal sclerosis [ <i>N</i> (%)]	3 (15.8)	3 (60.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

30 min. The peroxidase labeling was visualized with 0.2% 3,3'-diaminobenzidine (DAB) as chromogen. Sections were lightly counterstained with hematoxylin.

Semiquantitative assessment

TDP-43, tau, and A $\beta$  pathologies in the amygdala, anterior and posterior portions of the entorhinal cortex, hippocampal dentate gyrus, CA1, 2, 3, and 4 regions, subiculum, fusiform gyrus, occipitotemporal gyrus were semiquantitatively evaluated using the following grading system blinded to any clinical or pathological information:

1. The total number of TDP-43-positive neuronal cytoplasmic inclusions (NCIs) in each anatomical region was assessed as follows: – no lesion, + one inclusion, ++ two or three inclusions, +++ four or five inclusions, ++++ 6–10 inclusions, +++++ 11 or over inclusions. In addition, the presence or absence of neuronal intranuclear inclusions (NIIs) and dystrophic neurites was also assessed. Then, we classified the topographic distribution of TDP-43 pathological changes using following system, which is similar to that reported by Amador-Ortiz et al. [2]: the amygdala type: inclusions were present only in the amygdala; the limbic type: inclusions extend to the amygdala, hippocampal dentate gyrus, CA1-4, entorhinal cortex, and fusiform gyrus, but not in the occipitotemporal gyrus; the temporal type: inclusions are present in the limbic system and also the in the occipitotemporal gyrus.
2. Tau-positive neuronal inclusions were counted in low power microscopic fields: 0, no tau-positive lesions; 1, one neuronal inclusion per few microscopic fields; 2, one inclusion in every field; 3, 4–30 inclusions in every field; 4, over 30 inclusions associated with numerous neurites in every field.

3. A $\beta$  deposits were counted in low power microscopic fields: 0, no A $\beta$  deposits; 1, two to three A $\beta$  plaques in each field; 2, 4–10 A $\beta$  plaques in each field; 3, 11–20 A $\beta$  plaques in each field; 4, more than 20 A $\beta$  deposits in each field.

Hippocampal sclerosis (HS) was defined by neuronal loss with gliosis in the hippocampal CA1 and/or subiculum, with relatively preserved neurons in the CA4, 3, and two regions and absence of intracellular and extracellular NFTs, or ischaemic changes that might explain neuronal loss in the CA1 and subiculum. HS was assessed blind to any clinical or pathological information.

Statistical analysis

The Mann–Whitney *U* test and Fisher's exact test were used to compare the demographic and pathological data between TDP-43-positive and TDP-43-negative groups in PSP and CBD series, respectively. Correlations between ratings of TDP-43 pathology and demographic data, or ratings of tau and A $\beta$  pathologies in each anatomical region were assessed with Spearman's rank-order correlation statistic. Statistical analysis was performed using StatView for Macintosh program, version J-4.5. A value of *p* < 0.05 was accepted as significant.

Confocal laser scanning microscopy

Double-labeling immunofluorescence was performed with the combination of phosphorylation-dependent anti-TDP-43 (pAb pS409/410, rabbit, polyclonal, 1:1,000 [17]) and anti-tau antibodies (AT8, mouse, monoclonal, 1:500, Innogenetics, Ghent, Belgium). Sections from the amygdala and hippocampus in some PSP cases with TDP-43 pathology were pretreated by heating in a microwave oven for 5 min in 10 mM sodium citrate buffer, pH 6.0, at

100°C, allowed to cool then permeabilized with 0.2% (v/v) Triton X-100 in PBS. Following washing in PBS, non-specific antibody binding was blocked with normal sera and sections were incubated with a mixture of the two primary antibodies for 1 h at room temperature. After washing in PBS, sections were incubated with fluorescence-labeled secondary antibodies [AlexaFluor 488 anti-rabbit IgG (1:200) and AlexaFluor 555 anti-mouse IgG (1:200), Molecular Probes, Invitrogen, Paisley, UK]. After washing with PBS, sections were incubated with Toto-3 Iodide (Molecular Probes, Invitrogen, Paisley, UK) with 1 mg/ml RNase (Roche Diagnostics GmbH, Mannheim, Germany) at 37°C. To quench (lipofuscin) autofluorescence, sections were incubated in 0.1% Sudan Black B for 10 min at room temperature and washed with 0.1% Tx-PBS for 30 min. Sections were coverslipped with Vectashield mounting media (Vector Laboratories Inc., Burlingame, CA, USA). Images were collected on a Leica TCS SP5 AOBS upright confocal (Leica Microsystems, Milton Keynes, UK) using the 488 nm (19%), 543 nm (30%) and 633 nm (60%) laser lines, respectively. To eliminate cross-talk between channels, the images were collected sequentially.

#### Immunoblotting

Frozen tissue from the amygdala, hippocampus, and frontal, temporal, and occipital cortices in one PSP case with TDP-43 pathology, one FTLTDP case (as a positive control) and eight negative controls (six PSP, one LBD, and one pathologically normal case) were prepared for western blotting according to methods previously described by Neumann et al. [31]. Briefly, 1 g of fresh frozen brain was homogenized in 5 ml/g (w/v) of low salt (LS) buffer-containing 10 mM Tris pH 7.5, 5 mM EDTA pH 8.0, 1 mM DTT, 10% (w/v) sucrose and Roche complete EDTA-free protease inhibitor. Homogenates were sequentially extracted with increasing strength buffers [Triton X-100 buffer (LS buffer + 1% Triton X-100 + 0.5 M NaCl), Triton X-100 buffer with 30% sucrose to float myelin, Sarkosyl buffer (LS buffer + 1% *N*-lauroyl-sarcosine + 0.5 M NaCl)]. Detergent-insoluble pellets were extracted in 0.25 ml/g Urea buffer (7 M Urea, 2 M Thio-urea, 4% 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), 30 mM Tris-HCl pH 8.5, Roche complete EDTA free protease inhibitor. Prior to SDS-PAGE immunoblot analysis, urea fractions were added in 1:1 ratio to SDS sample buffer (10 mM Tris pH 6.8, 1 mM EDTA, pH 8.0, 40 mM DTT, 1% SDS, 10% Sucrose, 0.01% Bromophenol Blue). Protein was resolved on 12% Tris-Glycine SDS-PAGE gels along with size standard (Bio-Rad kaleidoscope broad-range marker; Bio-Rad, Hercules, CA, USA). Proteins were transferred onto

nitrocellulose membrane (Hybond ECL, GE Life Sciences, UK) and blocked for 1 h at 4°C in 5% (w/v) milk solution [5% powdered milk in Tris-buffered saline containing 0.1% Tween-20 (TBS-T)]. Membranes were incubated in phosphorylation-dependent mouse monoclonal antibody (mAb pS409/410, mouse, 1:1,000 [20]) for 1 h at room temperature followed by HRP-conjugated goat anti-mouse secondary antibody (Santa Cruz Biotechnology Inc, CA, USA). Antibodies were visualized by incubating in enhanced chemiluminescent reagent (ECL, GE Life Sciences) and imaged using the ImageQuant 350 system fitted with a F0,95 25 mm Fixed Lens (GE Healthcare, Life Sciences, UK). TDP-43 probed membranes were exposed for 5 min at different timeframes to obtain multiple images of differing intensity. Images were processed using ImageQuant TL software (GE Healthcare, Life Sciences, UK).

## Results

### Frequency and distribution of TDP-43 pathology

Clinical and pathological features for all subjects are shown in Table 1. TDP-43 pathology was noted in 5 of 19 PSP cases (26%) and in 2 of 12 CBD cases (17%). Disease duration, gender ratio and brain weight were not statistically different between PSP cases with and without TDP-43 pathology, or between CBD cases with and without TDP-43 pathology, respectively. Age at onset of disease (75 vs. 66 years) and age at death (82 vs. 74 years) tended to be higher, and dementia occurred more often, in PSP cases with TDP-43 pathology than in PSP cases without it (80 vs. 50%), although these differences did not reach statistical significance. One PSP case without TDP-43 pathology also had Lewy body pathology corresponding to brainstem-predominant type [26]. Ten PSP cases (3 TDP-43-positive and 7 TDP-43-negative cases) and four CBD cases (all were TDP-43-negative) had A $\beta$ -positive diffuse plaques in the amygdala, hippocampus, and/or temporal cortex. Of the three TDP-43-positive PSP cases, one case had only a few neuritic plaques in the occipitotemporal gyrus. None of the PSP or CBD cases in our series fit the pathological criteria of AD [9, 28, 39].

In PSP cases, TDP-43-positive NCIs were most frequently noted in the amygdala and dentate gyrus granule cells in the hippocampus (5 cases, 100% of TDP-43-positive PSP cases), followed by the anterior portion of the entorhinal cortex (4 cases, 80%), subiculum (3 cases, 60%), posterior portion of the entorhinal cortex (3 cases, 60%), occipitotemporal gyrus (2 cases, 50%), fusiform gyrus (2 cases, 40%), and CA1 region (2 cases, 20%) (Table 2, Fig. 1a–d). In addition to the rounded inclusions noted in FTLTDP, all PSP cases had many irregular shaped

**Table 2** Distribution of TDP-43 pathology in PSP and CBD cases

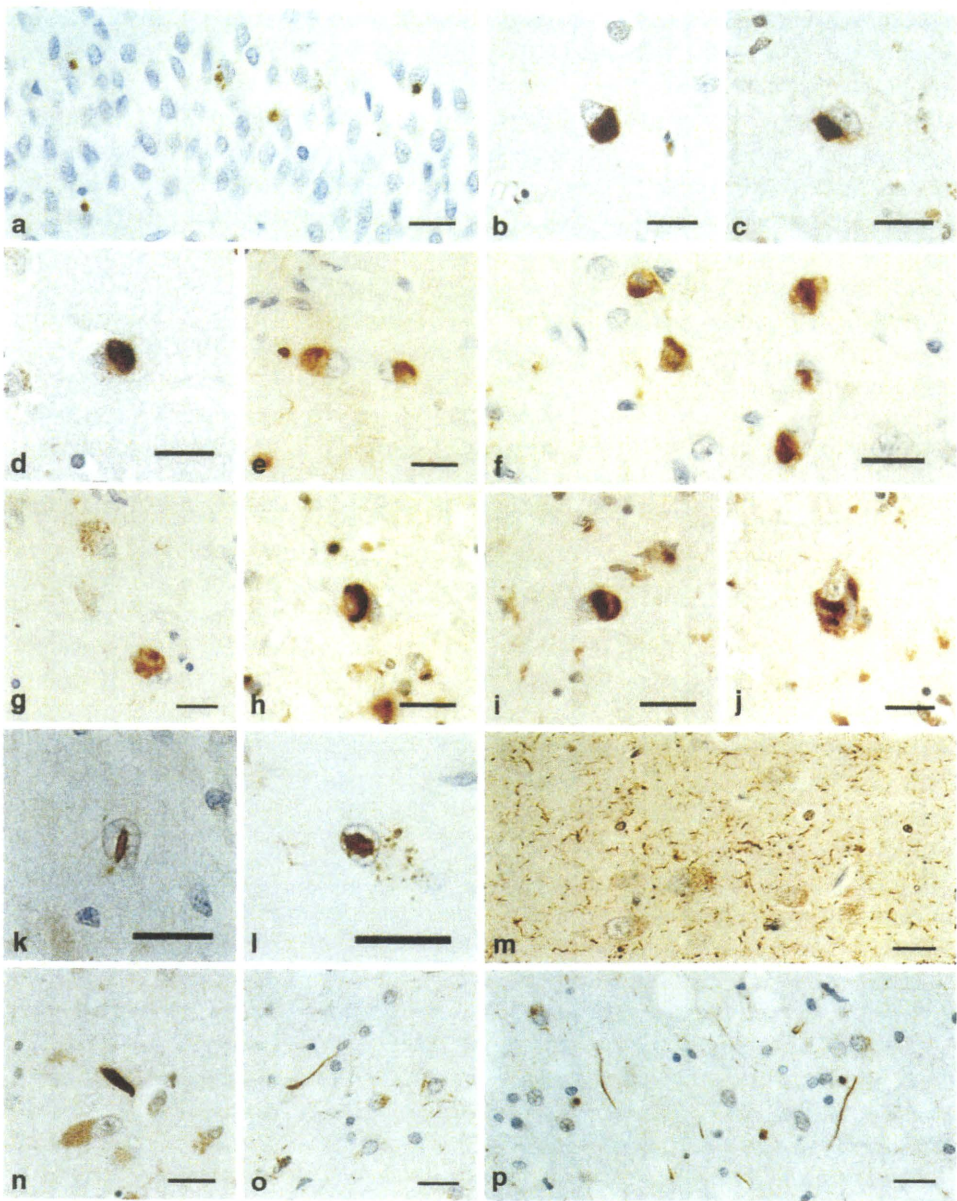
TDP-43 pathology											Argyrophilic grains <sup>b</sup>
No.	Amygdala	ant.EC	DG	CA3/4	CA2	CA1	SB	post.EC	FG	OTG	
TDP-43 distribution <sup>a</sup>											Hippocampal sclerosis (CA1/Subiculum)
PSP cases											
PSP1	++++	—	+	—	—	—	—	—	—	—	—
PSP2	+++++	+++++	+++++	—	—	—	+	—	—	n	+
PSP3	+++++	+++++	+++++	—	—	—	++	++++	—	—	—
PSP4	+++++	+++++	++	—	—	+	+++	+++++	+++++	+	+
PSP5	+++++	+++	+++++	—	—	++	—	++	+++	+++	+
%	100.0	80.0	100.0	0.0	0.0	20.0	60.0	60.0	40.0	50.0	60.0
CBD cases											
CBD1	+++	++	—	—	—	—	—	n.a.	n.a.	—	—
CBD2	+++++	++	++++	++	—	+++++	+++++	++	—	—	—
%	100.0	100.0	50.0	50.0	0.0	50.0	50.0	50.0	0.0	0.0	0.0

The stages of TDP-43 pathology: —, no lesion in the anatomical region; +, 1 inclusion in the anatomical region; ++, 2–3 inclusions in the anatomical region; ++++, 4–5 inclusions in the anatomical region; +++++, 6–10 inclusions in the anatomical region; ++++++, 11 or over inclusions in the anatomical region. The stage of hippocampal sclerosis: —, no; +, mild; ++, moderate; +++, severe. The stage of argyrophilic grains: —, absent; +, present. ant.EC, the anterior portion of the entorhinal cortex; DG, hippocampal dentate gyrus; SB, subiculum; post.EC, the posterior portion of the entorhinal cortex; FG, fusiform gyrus; OTG, occipitotemporal gyrus

<sup>a</sup> The amygdala type: inclusions were present only in the amygdala; the limbic type: inclusions extend to the limbic system, but not in the occipitotemporal gyrus; the temporal type: inclusions are present in the limbic system and occipitotemporal gyrus as well

<sup>b</sup> The distribution of argyrophilic grains are assessed using a staging system proposed by Saito et al. [35]

**Fig. 1** TDP-43-positive lesions in PSP. **a** Neuronal cytoplasmic inclusions (NCIs) in the hippocampal dentate gyrus. **b–d** NCIs in the entorhinal cortex. Irregular shaped NCIs in the entorhinal cortex (**e**), fusiform gyrus (**f**), and subiculum (**g**). These inclusions have weakly stained or unstained regions. Small dot-like structures are also seen in the neuronal cytoplasm (**g**). Horseshoe-shaped (**h**, **i**) and NFT-like (**j**) NCIs in the entorhinal cortex. Intranuclear inclusions in the amygdala (**k**) and in the subiculum (**l**), cases PSP3 and PSP2, respectively. **m** Massive short threads-like structures in the subiculum, case PSP3. **n** Thick, thread-like structures in the amygdala. **o**, **p** Long, thin thread-like structures in the amygdala. pAb pS409/410 immunohistochemistry. All scale bars 20  $\mu$ m



NCIs, such as flame-shape NFT-like, globose-type NFT-like, and horseshoe-like inclusions (Fig. 1e–j). One PSP case (PSP 2 in Table 2) showed a few NII in the subiculum (Fig. 1k, l). Two cases (PSP4 and PSP5) had abundant fine, short, thread-like structures immunopositive for TDP-43 from the CA1 to subiculum (Fig. 1m). TDP-43-positive thread-like structures were also observed in the amygdala (3 cases), entorhinal cortex (2 cases), CA1 (one case), and subiculum (one case) (Fig. 1n–p).

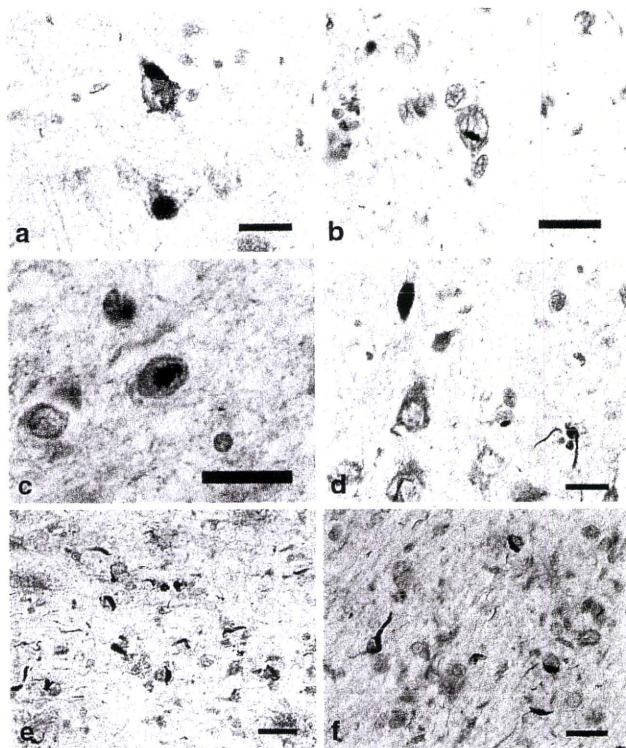
In two CBD cases, TDP-43-positive NCIs were observed in the amygdala, entorhinal cortex, hippocampal dentate gyrus, CA1, CA3/4, and subiculum (Table 2, Fig. 2a). The distribution of TDP-43 pathology was roughly consistent with that observed in PSP cases. NIIs were found in the subiculum and amygdala in one CBD case with severe,

TDP-43 pathology (Fig. 2b, c). Short thread-like structures immunopositive for TDP-43 were found in the amygdala, entorhinal cortex, CA1, CA3, and/or subiculum in both CBD cases with TDP-43 pathology. One CBD case had TDP-43-positive coiled body-like structures and thread-like structures in the alveus in the subiculum (Fig. 2d–f). Abnormal accumulation of TDP-43 was not found in the white matter of the temporal lobe and substantia nigra in any of the TDP-43-positive PSP or CBD cases.

#### Relationship between TDP-43 pathology and tau or A $\beta$ burden

The ratings for tau burden in the TDP-43-positive PSP cases tended to be higher (but not significantly so) than





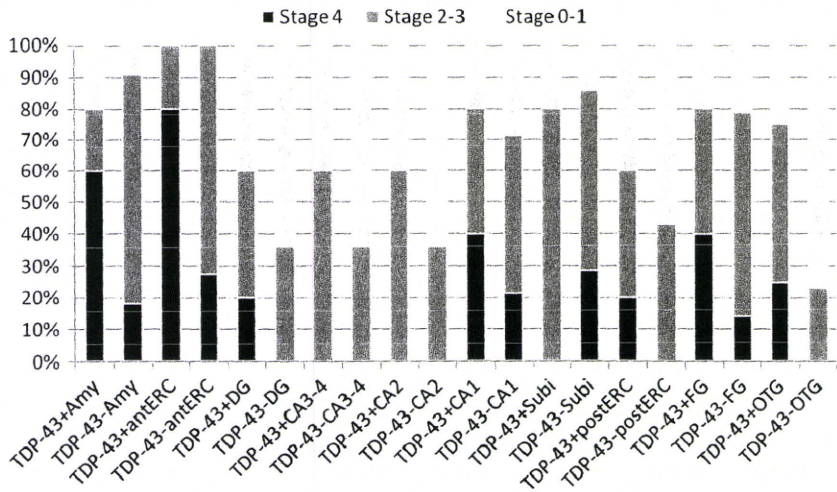
**Fig. 2** TDP-43-positive lesions in CBD. **a** Neuronal cytoplasmic inclusions (NCIs) in CA3 region of hippocampus. **b, c** Neuronal intranuclear inclusions in the amygdala. **d** A thick neurite and thin, thread-like structures in the amygdala. **e** Short thread-like structures and glial cytoplasmic inclusions (GCIs) in the alveus in the entorhinal cortex. **f** Coiled body-like structures and GCIs in the alveus in the entorhinal cortex. pAb pS409/410 immunohistochemistry. All scale bars 20 μm

those in the TDP-43-negative PSP cases, in almost all regions examined (i.e., including amygdala, entorhinal cortex, hippocampal dentate gyrus, CA1-4, fusiform gyrus, and occipitotemporal gyrus) (Fig. 3). In the PSP cases overall, rating for tau pathology in the occipitotemporal gyrus was significantly correlated with that of TDP-43 pathology ( $r = 0.504$ ,  $p < 0.05$ ), but no significant correlations between tau and TDP-43 ratings were found in any other regions. There were no significant differences in the degree of Aβ burden in any region between TDP-43-positive and TDP-43-negative PSP cases, and ratings for TDP-43 pathology did not correlate with those for Aβ burden in any region. Of three TDP-43-positive PSP cases having Aβ deposits, only one case had a few neuritic plaques in the occipitotemporal gyrus; however, this case did not have any TDP-43-positive inclusions in the region.

In the CBD cases, there were no significant differences in tau or Aβ burden in any region between TDP-43-positive and TDP-43-negative cases, and ratings for TDP-43 pathology did not correlate with those for tau or Aβ burden in any region.

#### Relationship of HS, argyrophilic grains, TDP-43 accumulation, and dementia

In 3 of 19 PSP cases (16%), evident neuronal loss in the CA1 and subiculum consistent with HS was noted (Fig. 4a, b). No CBD case showed HS. All three PSP cases with HS had a various degrees of TDP-43 pathology in the CA1 and/or subiculum (Fig. 4e–h), and two had extensive TDP-43



**Fig. 3** Tau burden in the limbic system in PSP cases with and without TDP-43 pathology. In all regions but the subiculum, tau burden in PSP cases with TDP-43 pathology is more severe than that in PSP cases without TDP-43 pathology. Stage 0–1, no to mild tau deposition; stages 2–3, moderate to severe tau deposition; stage 4,

very severe tau deposition (see detailed definition in the text). *TDP-43+* TDP-43-positive, *TDP-43–* TDP-43-negative, *Amy* amygdala, *anERC* the anterior portion of the entorhinal cortex, *DG* hippocampal dentate gyrus, *Subi* subiculum, *postERC* the posterior portion of the entorhinal cortex, *FG* fusiform gyrus, *OTG* occipitotemporal gyrus



**Fig. 4** Pathological features in the hippocampus in a PSP case with TDP-43, HS, and argyrophilic grains (PSP5).

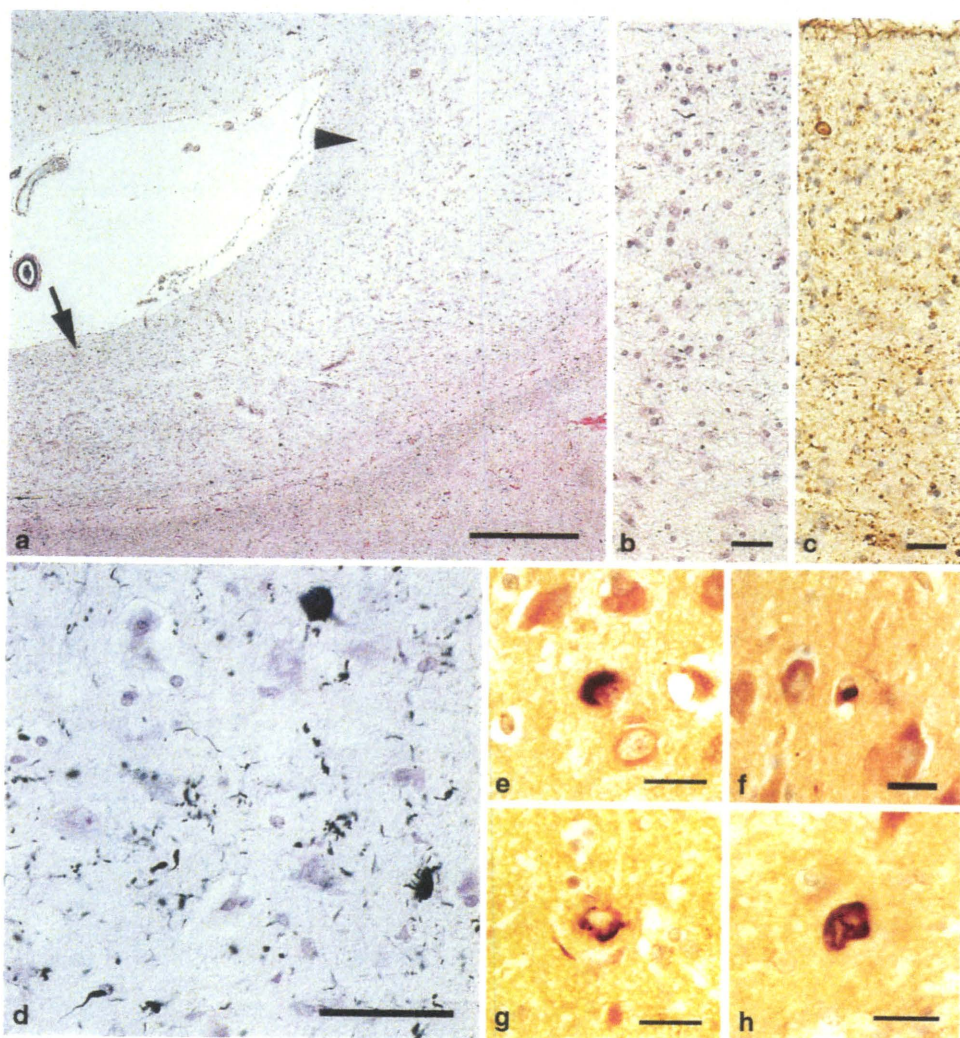
**a** A low power view of the hippocampal CA1 to subiculum. Severe reduction of the width with tissue rarefaction is noted in the subiculum (*arrow*) and to a lesser degree in the adjacent CA1 region (*arrowhead*).

**b** A moderate power view of the subiculum on the same section as that shown in **a**. Severe neuronal loss associated with gliosis is evident. Argyrophilic threads and grains are scattered, but tangles are rare.

**c** The subiculum on an adjacent section of **b**. A moderate number of tau-positive threads and grains, but only a few tangles, are seen. **d** Argyrophilic grains in CA1 region. **e, f** TDP-43-positive cytoplasmic inclusions in CA1 region.

**g** An irregular shaped TDP-43 accumulation in the subiculum. **h** A coiled body-like TDP-43-positive inclusion in the subiculum.

**a, b, d** Gallyas-Braak hematoxylin-eosin stain. **c** AT-8 immunohistochemistry. **e-h** pAb pS409/410 immunohistochemistry. Scale bars **a** 400  $\mu$ m, **b, c** 25  $\mu$ m, **d** 50  $\mu$ m, **e-h** 20  $\mu$ m



pathology in the limbic system: one case had both TDP-43 pathology and argyrophilic grains (Table 2). Two of the three PSP cases with HS had a few AT8-positive pretangles and argyrophilic grains in the CA1 and subiculum (Fig. 4b-d). Neurofibrillary tangles were rare in these regions in all PSP cases with HS (Fig. 4b). No significant ischemic changes in the hippocampal pyramidal neurons, or neuronal loss in the end plate, suggestive of a past history of severe epilepsy was noted in any of the PSP cases with HS. The frequency of HS in the TDP-43-positive PSP cases was significantly higher than that in TDP-43-negative PSP cases (60 vs. 0%,  $p = 0.021$ ). Dementia was present in all of the 3 TDP-43-positive PSP cases with HS (100%), 4 of the 5 TDP-43-positive PSP cases with and without HS (80%), 1 of 2 TDP-43-positive PSP cases without HS (50%), and 7 of 14 PSP cases lacking both (50%). The frequency of dementia was not significantly different between PSP cases with and without HS ( $p = 0.170$ ).

Concomitant argyrophilic grains were observed in four PSP (21%) and three CBD cases (25%) (Fig. 4d). Among

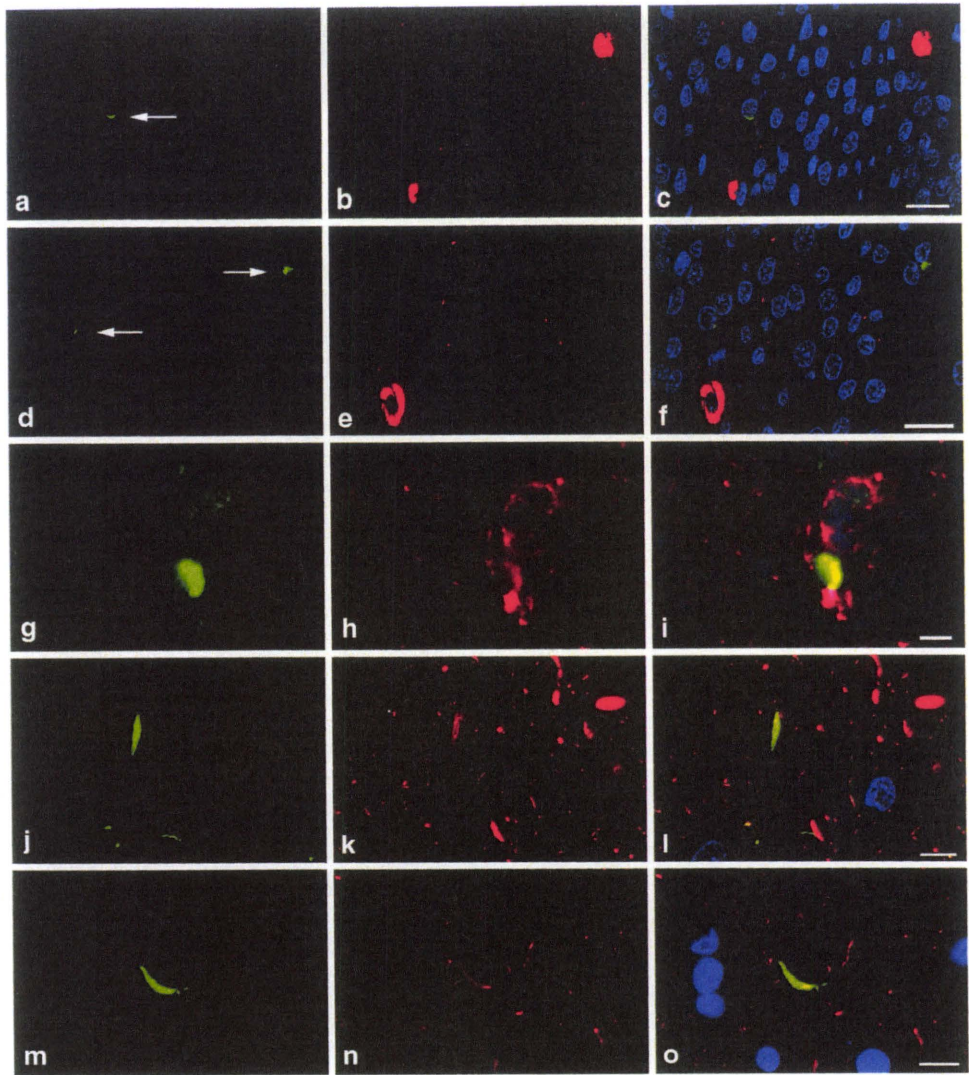
these cases, one PSP and one CBD case had TDP-43 pathology (Table 2, Fig. 4e-h). There was no significant difference in the frequency of argyrophilic grains between TDP-43-positive and TDP-43-negative PSP cases, or between CBD cases with and without TDP-43 pathology, respectively. However, in the TDP-43-positive PSP and CBD cases, argyrophilic grains were found in those cases with the most severe TDP-43 pathology (Table 2).

#### Double immunofluorescence labeling in PSP cases

In the PSP cases examined, TDP-43 and tau pathologies were independently present in the perikarya of granular cells in the hippocampal dentate gyrus with no coexistence of these proteins (Fig. 5a-f). In contrast, in the amygdala, TDP-43 accumulation was often intermingled with tau accumulation in NCIs and dystrophic neurites, and colocalization was frequent (Fig. 5g-o). In the entorhinal cortex and parahippocampal gyrus in one PSP case with argyrophilic grains, many tau-positive grain-like structures



**Fig. 5** Confocal double-immunofluorescence of TDP-43 (a, d, g, j, m) and tau (b, e, h, k, n) in PSP cases. Merged images are shown in c, f, i, l, and o. Blue fluorescence in merged images are nuclei. a–f In the hippocampal dentate gyrus, TDP-43 accumulation (arrows) is not colocalized with tau labeling. g–i In the amygdala, TDP-43 accumulation is often intermingled and colocalized with neuronal tau accumulation. j–o TDP-43-positive neurites (j, m) and many tau-positive neurites and granules (k, n) are seen in the amygdala. Coexistence of TDP-43 and tau is noted in some neurites (l, o). AT8 and pAb pS409/410 double immunofluorescence. Scale bars a–c 25  $\mu$ m, d–f 25  $\mu$ m, g–i 2.5  $\mu$ m, j–l 7.5  $\mu$ m, m–o 7.5  $\mu$ m



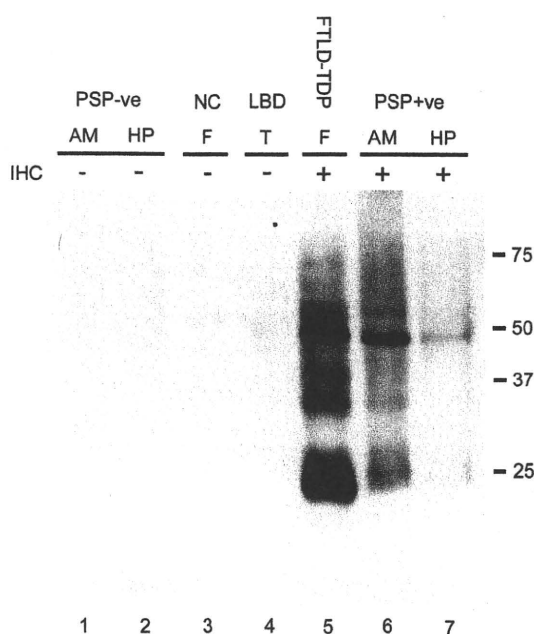
were demonstrated, and TDP-43 was colocalized with tau in some of these structures (data not shown).

#### Biochemical analyses of TDP-43 in PSP cases

Immunoblot analysis of the sarkosyl-insoluble, urea-soluble fraction with mAb pS409/410 demonstrated distinct bands at (approximately) 45 and 25 kDa, as well as high molecular weight smears in the amygdala of a PSP case having TDP-43 pathology (Fig. 6, lane 6) and in the frontal cortex of a FTLD-TDP case (lane 5). Weak 25 and 45 kDa bands were also observed in the hippocampus in a PSP case, which had very mild TDP-43 pathology at this site (lane 7). Pathological TDP-43 bands and smear were not demonstrated in any of the other cases lacking TDP-43 pathology, including those with PSP (lanes 1 and 2) or Lewy body disease (lane 4), or in normal control cases (lane 3).

#### Discussion

This is the first study demonstrating abnormal accumulations of phosphorylated TDP-43 in the limbic system in a significant proportion (26%) of patients with PSP. Immunoblot analysis also demonstrated biochemical alterations in TDP-43 in tissue samples from a PSP case with TDP-43 pathology, similar to those in FTLD-TDP and ALS. Regional tau burden in PSP cases with TDP-43 pathology was higher than that in PSP cases without it, and TDP-43 burden was significantly correlated with that of tau in the occipitotemporal cortex. The frequency of HS in PSP cases with TDP-43 pathology was significantly higher than that in PSP cases without it. Collectively, these findings suggest that (1) PSP is one of the tauopathies in which pathological TDP-43 accumulation can occur in the limbic system, and (2) TDP-43 pathology may be associated with the occurrence of HS in PSP cases.



**Fig. 6** Immunoblot analysis of the sarkosyl-insoluble fraction in representative PSP cases with phosphorylation-dependent monoclonal anti-TDP-43 antibody (mAb pS409/410). The 45 kDa full length TDP-43, 25 kDa fragments, and high molecular weight smear are strongly labeled in the amygdala of a PSP case with TDP-43 pathology (lane 6) and in the frontal cortex of a FTLD-TDP case (lane 5). Weakly stained 45 and 25 kDa bands are noted in the hippocampus of a PSP case (lane 7), in which TDP-43 pathology was mild. Similar 45 and 25 kDa bands and smears were not immunolabeled in any of the other cases without detectable TDP-43 pathology by immunohistochemistry (lanes 1–4). Normal 43 kDa TDP-43 is not stained by this phosphorylation-dependent antibody in any case. PSP progressive supranuclear palsy, LBD Lewy body disease, NC normal control, AM amygdala, HP hippocampus, F frontal cortex, T temporal cortex, IHC pAb pS409/410 immunohistochemistry

Previous studies have demonstrated variable frequencies of concurrent TDP-43 pathology in many tauopathies: 23–56% in AD cases [2, 4, 40], 31–60% in DLB + AD cases [4, 30], 15% in CBD cases [40], and 60% in AGD cases [14]. Why no cases of PSP with TDP-43 pathology have previously been described is not clear. Our present findings show that, at least some, PSP cases may share a common pathophysiological background involving TDP-43 accumulation with other tauopathies with TDP-43 pathology. Several studies demonstrated that concurrent AD-type pathology was associated with the development of TDP-43 pathology in some neurodegenerative diseases [2, 4, 7, 14, 30]. However, it was unlikely that the development of TDP-43 pathology in our PSP series can be explained by the influence of A $\beta$  deposits or neuritic plaques. For example, of all ten PSP cases having A $\beta$  deposits, nine cases had only diffuse plaques, and the degree of A $\beta$  deposition was not significantly different between TDP-43-positive and TDP-43-negative PSP cases and was not correlated with that of TDP-43 pathology in any regions.

Although only one PSP case had a few neuritic plaques in the occipitotemporal gyrus, no TDP-43-positive inclusion was noted in the region.

Our findings are inconsistent with previous studies that failed to demonstrate immunohistochemical or biochemical abnormalities of TDP-43 in PSP cases [2, 3, 18, 40]. Considering that the sample size investigated in one of these previous studies [40] was far larger than that in our own study, the most plausible cause of the discrepancy may be the difference of the sensitivities of anti-TDP-43 antibodies employed: phosphorylation-dependent anti-TDP-43 antibodies do not stain normal nuclei, making true TDP-43-positive inclusions more readily identifiable [17, 37]. The distribution of TDP-43 pathology observed in our PSP cases was very similar to that reported previously in AD [2, 4, 18, 19, 40], DLB + AD [4, 30], and CBD [40], but tended to be more restricted than that in ALS/PDC of Guam [15, 16, 27]. Most frequently affected sites in these tauopathies are the amygdala and hippocampal dentate gyrus. Given these findings, it is plausible that the frequent TDP-43 accumulation in these sites in tauopathies is associated with some region-specific, rather than disease-specific, mechanism. On the other hand, it remains unclear whether TDP-43 is abnormally accumulated through an identical pathophysiological mechanism in various anatomical regions. For example, it was reported that abnormal TDP-43 accumulation was significantly correlated with the severity of tau pathology in AD cases [4] and Lewy body disease including many DLB + AD cases [30]. This same statistical relationship was observed in our PSP cases. Furthermore, in our present studies, TDP-43 was often colocalized with tau in NCIs and dystrophic neurites in the amygdala, although there were also TDP-43-positive but tau-negative lesions in this site. A coexistence of TDP-43 and tau in the same neuron in the amygdala and temporal cortex was also reported in AD and DLB cases in previous studies [4, 18]. However, in contrast to the amygdala, a coexistence of TDP-43 and tau in the same neuron in the hippocampal dentate gyrus was not seen in our PSP cases. This trend regarding non-colocalization of these two proteins was also noticed in the dentate granular cells in AD [40] and AGD brains [14]. This suggests that the mechanism underlying the accumulation of TDP-43 is different at least between the amygdala and hippocampal dentate gyrus, or that there is some unknown factor that can influence the occurrence of both TDP-43 and tau pathologies. In addition, considering the potential relationship between tau and TDP-43 in PSP presented in this paper, whether TDP-43 pathology is also noted in several other regions that are often involved by tau-associated lesions (e.g., the frontal cortex and basal ganglia) needs to be investigated in the future studies.



There is little known about the relationship between PSP and HS. In our series, 3 of 19 PSP cases (16%) had evident neuronal loss in the CA1 and/or subiculum consistent with the definition of HS. Furthermore, all of the cases with HS had TDP-43 pathology, and one of the three cases also had argyrophilic grains. It has been reported that HS cases have variable underlying pathologies, including the 'pure form' of HS [1, 21, 34], FTLN-TDP [23], FTLN with motor neuron disease [29], AD [2], CBD [36], DLB [13], and AGD [5, 13]. Present findings support the possibility that the development of HS, at least in some PSP cases, may occur in association with concurrent TDP-43 pathology. On the other hand, whether the development of HS in PSP cases is correlated with the severity of tau or TDP-43 pathology remains unclear. Considering the relatively small size of the samples examined in the present study, the relationship between HS and TDP-43 accumulation in PSP, as well as the frequencies of these pathological features, needs to be confirmed in a larger case series.

Although influence of concurrent TDP-43 pathology on clinical features in tauopathies is not fully understood, some previous studies in AD, have demonstrated a comorbidity such that a concomitant TDP-43 pathology was associated with a later age at onset and death [4, 24], and significantly poorer cognitive function [24]. On the other hand, a study investigating a relatively small series of AGD did not demonstrate any significant difference in the age at death or disease duration between cases with and without TDP-43 pathology [14]. It is known that patients with PSP frequently exhibit psychiatric and behavioral disturbances, and that cognitive decline in PSP is associated with the atrophy in the orbitofrontal cortex [11] and more severe tau burden in the neocortex and hippocampus [6, 8, 22]. More recently, it was also reported that clinical presentation, including the occurrence of dementia, is influenced by the distribution and severity of tau pathology [42, 43]. In our PSP series, although not statistically significantly, the frequency of dementia in PSP cases with both TDP-43 and HS (100%), and that in all PSP cases with TDP-43 pathology (80%), were higher than that in PSP cases lacking both (50%). The potential co-morbid effect of concurrent TDP-43 pathology and/or HS on cognitive impairment in patients with PSP needs to be explored by further clinicopathological studies.

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## References

1. Ala TA, Beh GO, Frey WH 2nd (2000) Pure hippocampal sclerosis: a rare cause of dementia mimicking Alzheimer's disease. *Neurology* 54:843–848
2. Amador-Ortiz C, Lin WL, Ahmed Z et al (2007) TDP-43 immunoreactivity in hippocampal sclerosis and Alzheimer's disease. *Ann Neurol* 61:435–445
3. Arai T, Hasegawa M, Akiyama H et al (2006) TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochem Biophys Res Commun* 351:602–611
4. Arai T, Mackenzie IR, Hasegawa M et al (2009) Phosphorylated TDP-43 in Alzheimer's disease and dementia with Lewy bodies. *Acta Neuropathol* 117:125–136
5. Beach TG, Sue L, Scott S et al (2003) Hippocampal sclerosis dementia with tauopathy. *Brain Pathol* 13:263–278
6. Bigio EH, Brown DF, White CL 3rd (1999) Progressive supranuclear palsy with dementia: cortical pathology. *J Neuropathol Exp Neurol* 58:359–364
7. Bigio EH, Mishra M, Hatanpaa KJ et al (2010) TDP-43 pathology in primary progressive aphasia and frontotemporal dementia with pathologic Alzheimer disease. *Acta Neuropathol* (in press). doi: 10.1007/s00401-010-0681-2
8. Braak H, Braak E (1990) Neurofibrillary changes confined to the entorhinal region and an abundance of cortical amyloid in cases of presenile and senile dementia. *Acta Neuropathol* 80:479–486
9. Braak H, Alafuzoff I, Arzberger T, Kretschmar H, Del Tredici K (2006) Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. *Acta Neuropathol* 112:389–404
10. Cairns NJ, Neumann M, Bigio EH et al (2007) TDP-43 in familial and sporadic frontotemporal lobar degeneration with ubiquitin inclusions. *Am J Pathol* 171:227–240
11. Cordato NJ, Duggins AJ, Halliday GM, Morris JG, Pantelis C (2005) Clinical deficits correlate with regional cerebral atrophy in progressive supranuclear palsy. *Brain* 128:1259–1266
12. Davidson Y, Kelley T, Mackenzie IR et al (2007) Ubiquitinated pathological lesions in frontotemporal lobar degeneration contain the TAR DNA-binding protein, TDP-43. *Acta Neuropathol* 113:521–533
13. Dickson DW, Davies P, Bevona C et al (1994) Hippocampal sclerosis: a common pathological feature of dementia in very old (> or = 80 years of age) humans. *Acta Neuropathol* 88:212–221
14. Fujishiro H, Uchikado H, Arai T et al (2009) Accumulation of phosphorylated TDP-43 in brains of patients with argyrophilic grain disease. *Acta Neuropathol* 117:151–158
15. Geser F, Winton MJ, Kwong LK et al (2008) Pathological TDP-43 in parkinsonism-dementia complex and amyotrophic lateral sclerosis of Guam. *Acta Neuropathol* 115:133–145
16. Hasegawa M, Arai T, Akiyama H et al (2007) TDP-43 is deposited in the Guam parkinsonism-dementia complex brains. *Brain* 130:1386–1394
17. Hasegawa M, Arai T, Nonaka T et al (2008) Phosphorylated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Ann Neurol* 64:60–70
18. Higashi S, Iseki E, Yamamoto R et al (2007) Concurrence of TDP-43, tau and alpha-synuclein pathology in brains of Alzheimer's disease and dementia with Lewy bodies. *Brain Res* 1184:284–294
19. Hu WT, Josephs KA, Knopman DS et al (2008) Temporal lobar predominance of TDP-43 neuronal cytoplasmic inclusions in Alzheimer disease. *Acta Neuropathol* 116:215–220

20. Inukai Y, Nonaka T, Arai T et al (2008) Abnormal phosphorylation of Ser409/410 of TDP-43 in FTLD-U and ALS. *FEBS Lett* 582:2899–2904
21. Jellinger KA (2000) Pure hippocampal sclerosis: a rare cause of dementia mimicking Alzheimer's disease. *Neurology* 55:739–740
22. Jellinger KA (2008) Different tau pathology pattern in two clinical phenotypes of progressive supranuclear palsy. *Neurodegener Dis* 5:339–346
23. Josephs KA, Dickson DW (2007) Hippocampal sclerosis in tau-negative frontotemporal lobar degeneration. *Neurobiol Aging* 28:1718–1722
24. Josephs KA, Whitwell JL, Knopman DS et al (2008) Abnormal TDP-43 immunoreactivity in AD modifies clinicopathologic and radiologic phenotype. *Neurology* 70:1850–1857
25. Kabashi E, Valdmanis PN, Dion P et al (2008) TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis. *Nat Genet* 40:572–574
26. McKeith IG, Dickson DW, Lowe J et al (2005) Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. *Neurology* 65:1863–1872
27. Miklosy J, Steele JC, Yu S et al (2008) Enduring involvement of tau,  $\beta$ -amyloid,  $\alpha$ -synuclein, ubiquitin and TDP-43 pathology in the amyotrophic lateral sclerosis/parkinsonism–dementia complex of Guam (ALS/PDC). *Acta Neuropathol* 116:625–637
28. Mirra SS, Heyman A, McKeel D et al (1991) The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology* 41:479–486
29. Nakano I (1993) Temporal lobe lesions in amyotrophic lateral sclerosis with or without dementia—a neuropathological study. *Neuropathology* 13:215–227
30. Nakashima-Yasuda H, Uryu K et al (2007) Co-morbidity of TDP-43 proteinopathy in Lewy body related diseases. *Acta Neuropathol* 114:221–229
31. Neumann M, Sampathu DM, Kwong LK et al (2006) Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* 314:130–133
32. Neumann M, Mackenzie IR, Cairns NJ et al (2007) TDP-43 in the ubiquitin pathology of frontotemporal dementia with VCP gene mutations. *J Neuropathol Exp Neurol* 66:152–157
33. Obi K, Akiyama H, Kondo H et al (2008) Relationship of phosphorylated  $\alpha$ -synuclein and tau accumulation to Abeta deposition in the cerebral cortex of dementia with Lewy bodies. *Exp Neurol* 210:409–420
34. Probst A, Taylor KI, Tolnay M (2007) Hippocampal sclerosis dementia: a reappraisal. *Acta Neuropathol* 114:335–345
35. Saito Y, Ruberu NN, Sawabe M et al (2004) Staging of argyrophilic grains: an age-associated tauopathy. *J Neuropathol Exp Neurol* 63:911–918
36. Schneider JA, Watts RL, Gearing M et al (1997) Corticobasal degeneration: neuropathologic and clinical heterogeneity. *Neurology* 48:959–969
37. Schwab C, Arai T, Hasegawa M, Yu S, McGeer PL (2008) Colocalization of transactivation-responsive DNA-binding protein 43 and huntingtin in inclusions of Huntington disease. *J Neuropathol Exp Neurol* 67:1159–1165
38. Sreedharan J, Blair IP, Tripathi VB et al (2008) TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. *Science* 319:1668–1672
39. The National Institute on Aging, and Reagan Institute Working Group (1997) Consensus recommendations for the postmortem diagnosis of Alzheimer disease. The National Institute on Aging, and Reagan Institute Working Group on diagnostic criteria for the neuropathological assessment of Alzheimer disease. *Neurobiol Aging* 18:S1–S2
40. Uryu K, Nakashima-Yasuda H, Forman MS et al (2008) Concomitant TAR-DNA-binding protein 43 pathology is present in Alzheimer disease and corticobasal degeneration but not in other tauopathies. *J Neuropathol Exp Neurol* 67:555–564
41. Van Deerlin VM, Leverenz JB, Bekris LM et al (2008) TARDBP mutations in amyotrophic lateral sclerosis with TDP-43 neuropathology: a genetic and histopathological analysis. *Lancet Neurol* 7:409–416
42. Williams DR, Lees AJ (2009) Progressive supranuclear palsy: clinicopathological concepts and diagnostic challenges. *Lancet Neurol* 8:270–279
43. Williams DR, Holton JL, Strand C et al (2007) Pathological tau burden and distribution distinguishes progressive supranuclear palsy-parkinsonism from Richardson's syndrome. *Brain* 130:1566–1576
44. Yokoseki A, Shiga A, Tan CF et al (2008) TDP-43 mutation in familial amyotrophic lateral sclerosis. *Ann Neurol* 63:538–542



## □ CASE REPORT □

## TDP-43 M337V Mutation in Familial Amyotrophic Lateral Sclerosis in Japan

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### Abstract

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The clinical features of a Japanese family with autosomal dominant adult-onset amyotrophic lateral sclerosis (ALS) are reported. Weakness initially affected the bulbar musculature, with later involvement of the extremities. Genetic studies failed to detect any mutations of the Cu/Zn superoxide dismutase-1 (SOD1) and Dynactin1 (DCTN1) genes, but revealed a single base pair change from wild-type adenine to guanine at position 1009 in TAR-DNA-binding protein (TDP-43), resulting in a methionine-to-valine substitution at position 337. The immunohistochemical study on autopsied brain of the proband's aunt showed TDP-43-positive cytoplasmic inclusions in the anterior horn cells of the spinal cord and in the hypoglossal nucleus, as well as glial cytoplasmic inclusions in the precentral gyrus, suggesting that a neuroglial proteinopathy was related to TDP-43. In conclusion, a characteristic clinical phenotype of familial ALS with initial bulbar symptoms occurred in this family with TDP-43 M337V substitution, the pathomechanism of which should be elucidated.

**Key words:** Amyotrophic lateral sclerosis (ALS), TAR-DNA-binding protein 43 (TDP-43)

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### Introduction

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Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disorder that is characterized pathologically by the degeneration of motor neurons in the brain and spinal cord, and clinically by progressive weakness and death within a few years of onset. Recently, TAR DNA-binding protein 43 (TDP-43) was identified as the major pathological protein in the motor neuron inclusions found in sporadic ALS and superoxide dismutase 1 (SOD1)-negative familial ALS, as well as in frontotemporal lobar degeneration with ubiquitin-immunoreactive, tau-negative inclusions (FTLD-U). Although the role of TDP-43 in the pathogenesis of these neurodegenerative disorders remains to be elucidated, several mutations of TDP-43 have been identified in individuals with sporadic and familial ALS, sug-

gesting that TDP-43 may be a causative protein for these disorders (1-6). Here we first report the detailed clinical features of affected members of a Japanese family who suffered from ALS linked to TDP-43 M337V mutation.

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### Case Report

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The proband (III-2 in Fig. 1-1) was a Japanese woman aged 61 years. She developed dysarthria at the age of 55 years, which became progressively worse. One year later, she also noted dysphagia. Neurological examination at the age of 56 revealed minimal atrophy of the facial muscles and tongue, markedly diminished reflexes of the palatal and pharyngeal muscles, and slow movements and minimal fasciculation of the tongue. Her deep tendon reflexes, including the jaw jerk, were highly exaggerated. At the age of 57, her dysphagia worsened, and atrophy and fasciculation of the

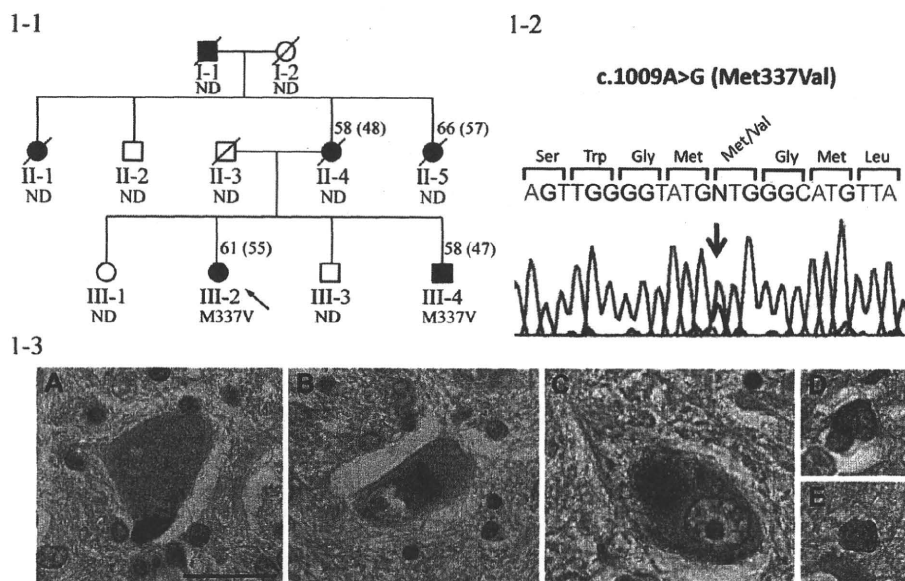
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**Figure 1.** 1-1. Pedigree of the present family. Circles represent women and squares represent men. The slashed symbols indicate deceased subjects. Known affected persons are shown as filled symbols. The arrow represents the proband. Age at death or current age and age at disease onset in parenthesis are indicated. ND=not determined. 1-2. Chromatogram of Patient III-2 (the proband). Chromatogram shows the heterozygous sequence trace of A to G for genotyping by the reverse primer. The nucleotide position of substitution is indicated by arrow. 1-3. Immunocytochemical findings in Patient II-5. TDP-43 positive cytoplasmic inclusions in the anterior horn of the spinal cord (A, B) and in the hypoglossal nucleus (C). Glial cytoplasmic inclusions in the precentral gyrus (D, E). (A, C) Phosphorylation-independent anti-TDP-43 antibody; (B, D, E) phosphorylation-dependent anti-TDP-43 antibody (pS409/410). The sections were counterstained with hematoxylin to reveal nuclei. Bar in A=25 μm.

tongue became more prominent. Muscle weakness of the lower extremities showed slow progression, predominantly in the distal regions. At the age of 58, she was almost unable to protrude her tongue. At the age of 61, she also noted mild weakness of the upper extremities. Needle EMG showed marked neurogenic changes of the biceps, abducens pollicis brevis, vastus lateralis and tibialis anterior muscles of the right side, as well as a mild neurogenic pattern in her right masseter.

The aunt of the proband (II-5 in Fig. 1-1), a Japanese woman, developed dysarthria at the age of 57 years, followed by dysphagia, weakness of the upper extremities, and difficulty with breathing. She could walk without support until her death at the age of 66. The results of the neuropathological examination were reported in detail (7).

The younger brother of the proband (III-4 in Fig. 1-1), a Japanese man, developed dysarthria at the age of 44 years. Neurological examination at the age of 47 showed slight dysarthria, poor movement of the soft palate, exaggerated pharyngeal reflexes and jaw jerk, slow movements, slight atrophy and fasciculation of the tongue. These findings were mainly related to pseudobulbar palsy. He also showed hyperreflexia in the upper and lower extremities (predominantly in the latter) without any pathological reflexes. Needle

EMG revealed neurogenic changes of the masseter and orbicularis oris muscles, while there was a normal pattern in the tongue and extremities. He had no dysphagia, muscle weakness, or atrophy of the upper and lower extremities, as well as no sensory disturbance or vesicorectal disturbance. He could stand and walk unaided. His condition deteriorated slowly and progressively over the next 10 years. At present, he is 58 years old and virtually bed-ridden with a gastrostomy and minimal communication. Patient II-4, Patient II-1 and Patient I-1 all suffered from dysarthria until death, the details of which were unknown.

The present family demonstrated autosomal dominant inheritance of ALS and both sexes were affected. Six family members (patients I-1, II-1, II-4, II-5, III-2 and III-4) were suspected to have ALS, among whom three (II-5, III-2 and III-4) had definite ALS according to the El-Escorial criteria. All six patients (2 men and 4 women) with familial ALS in this family showed dysarthria at the onset, so their clinical courses were indistinguishable from bulbar-onset ALS. There was no history of dementia and no atypical features in the kindred. Based on the information of the patients with good clinical records (patients II-4, II-5, III-2 and III-4), the mean age of symptom onset was 52.5 years (range 44-61 years) and the mean disease duration was 9.5 years (range

9-10 years) from symptom onset to death based on the outcome in patients II-4 and II-5.

After approval by the Ethics Committees of all participating institutions, sequencing of the coding regions of the TDP-43 gene in the patients (III-2 and III-4) was performed, which showed a heterozygous A-to-G transition at cDNA position 1009 (c.1009A>G) resulting in a methionine-to-valine substitution at position 337 (M337V) in a highly conserved region of exon 6 (Fig. 1-2). None of the control 1,621 healthy subjects providing informed consent had this missense mutation.

Immunohistochemistry analysis of the brain of patient II-5 using both a phosphorylation-independent anti-TDP-43 antibody (10782-2-AP) and a phosphorylation-dependent anti-TDP-43 antibody (pS409/410) (8) showed neuronal cytoplasmic inclusions in the anterior horn of the spinal cord (Fig. 1-3A, B) and the hypoglossal nucleus (Fig. 1-3C), as well as glial cytoplasmic inclusions in the precentral gyrus (Fig. 1-3D, E).

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## Discussion

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In the present study, we detected the M337V substitution in TDP-43 in a Japanese family with ALS, including one case confirmed at autopsy (patient II-5). We consider that this M337V substitution was associated with the disease, since M337V was present in two affected individuals from one generation and never in the control subjects, in addition to the fact that M337V substitution of TDP-43 has already been reported to segregate with ALS within two probably unrelated kindreds (2, 6). In a UK autosomal dominant ALS family carrying M337V substitution of TDP-43 reported by Sreedharan et al (2), three had limb-onset ALS and two had bulbar-onset ALS. The mean age of symptom onset was 47 years (range 44 to 52). Mean disease duration was 5.5 years (range 4 to 7) from symptom onset to death. The M337V mutation carrier in a US family with a strong family history of ALS reported by Rutherford et al (6) showed upper limb-onset ALS at 38 years of age, 6 years younger than the earliest onset age reported in the British M337V family (2). In the present paper, we show the first Japanese family with ALS carrying M337V substitution of TDP-43, in which virtually all patients showed dysarthria at the onset, suggesting

that their clinical courses were indistinguishable from bulbar-onset ALS. Among these UK, US and Japanese families carrying TDP-43 M337V mutation, the common features include no signs of dementia or other atypical features of ALS and past middle age onset of the disease. However, the signs at onset were different among these three families, and mean disease duration in the present Japanese family was longer than that in the UK family, indicating the phenotype of this mutation is quite variable. The identification of M337V in three genealogically unrelated ALS families further implies the pathogenicity of TDP-43 M337V mutation.

Regarding the pathogenicity of TDP-43 M337V mutation, Sreedharan et al (2) reported that mutant forms of TDP-43 (including M337V) fragmented *in vitro* more easily than wild-type TDP-43 and, *in vivo*, caused neuronal apoptosis and developmental delay in chick embryos, suggesting a pathophysiological link between TDP-43 and ALS. In addition, Rutherford et al (6) showed that biochemical analysis of TDP-43 in lymphoblastoid cell lines of carriers with TDP-43 mutations including M337V revealed a substantial increase in fragments possibly cleaved by caspase, including the ~25 kDa fragment, compared to control cell lines, supporting TDA-43 as a cause of ALS. Our immunohistochemical study showed TDP-43 positive cytoplasmic inclusions in the anterior horn cells of the spinal cord and in the hypoglossal nucleus, as well as glial cytoplasmic inclusions in the precentral gyrus, suggesting that a neuroglial proteinopathy was related to TDP-43. Further investigations including biochemical analysis using patients' fibroblasts or lymphoblastoid cells will be necessary to elucidate the mechanism by which TDP-43 contributes to ALS and to develop new drugs that block the pathological process related to TDP-43.

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## References

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1. Gitcho MA, Baloh RH, Chakraverty S, et al. TDP-43 A315T mutation in familial motor neuron disease. *Ann Neurol* **63**: 535-538, 2008.
2. Sreedharan J, Blair IP, Tripathi VB, et al. TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. *Science* **319**: 1668-1672, 2008.
3. Yokoseki A, Shiga A, Tan CF, et al. TDP-43 mutation in familial amyotrophic lateral sclerosis. *Ann Neurol* **63**: 538-542, 2008.
4. Kabashi E, Valdmanis PN, Dion P, et al. TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis. *Nat Genet* **40**: 572-574, 2008.
5. Van Deerlin VM, Leverenz JB, Bekris LM, et al. TARDBP mutations in amyotrophic lateral sclerosis with TDP-43 neuropathology: a genetic and histopathological analysis. *Lancet Neurol* **7**: 409-416, 2008.
6. Rutherford NJ, Zhang YJ, Baker M, et al. Novel mutations in TARDBP (TDP-43) in patients with familial amyotrophic lateral sclerosis. *PLoS Genetics* **4**: e1000193, 2008.
7. Tsuchiya K, Shintani S, Nakabayashi H, et al. Familial amyotrophic lateral sclerosis with onset in bulbar sign, benign clinical course, and Bunina bodies: a clinical, genetic, and pathological study of a Japanese family. *Acta Neuropathol* **100**: 603-

607, 2000.

8. Hasegawa M, Arai T, Nonaka T, et al. Phosphorylated TDP-43 in

frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Ann Neurol* **64**: 60-70, 2008.

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原著

紀伊半島南部地域における筋萎縮性側索硬化症  
——和歌山県内多発地域における最近の発症率の推移と臨床像の変化

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## 紀伊半島南部地域における筋萎縮性側索硬化症 ——和歌山県内多発地域における最近の発症率の推移と臨床像の変化

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### Changes in the Incidence and Clinical Features of ALS in the Koza, Kozagawa, and Kushimoto Area of the Kii Peninsula — From the 1960s to the 2000s Follow-up Study

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#### Abstract

In the 1960's, ALS was highly prevalent in the southern part of the Kii Peninsula, especially in the Koza, Kozagawa, and Kushimoto area (K area). Thereafter, the incidence of ALS was considered to have gradually decreased, and the disease almost disappeared in the 1980's. However, new patients have been continuously identified in this area, and indicating the importance of studying the changes in the incidences of ALS. This study investigated the characteristic clinical features and incidence of ALS in K area during the following periods: period I: between 1967 and 1971, period II: between 1989 and 1999, and period III: between 2000 and 2008. Data on all patients with motor neuron disease were collected for each year within these periods from medical doctors and medical staff of the regional public health center and municipal office. Neurologists on our research team examined and assessed each of these patients on the basis of the El Escorial criteria. Probable and definite ALS patients diagnosed by neurologists using the El Escorial criteria in K area during the research periods were collected. The crude incidence rate of ALS in K area were similar in period I, i.e. 6.0/100,000, and in period III, i.e., 5.7/100,000. The age-, and sex-adjusted incidence (considering the 2000 census) in women in K area during period III, especially in Kozagawa district, was higher than that in periods I and II. The adjusted incidence rate in Kozagawa district was 8.8/100,000, and was higher than that in other areas of the world. The clinical features of patients in this area were quite similar to those of patients with classical ALS. Five patients from 3 families with a family history of ALS and 2 patients without a family history presented with the clinical features of ALS and PDC during these research periods. The mean age at onset for period III was higher than that in period I ( $p < 0.01$ ). The frequency of ALS patients with upper-extremity onset in period III was lower than that in period I ( $p = 0.05$ ), whereas the frequency of patients with bulbar-onset has recently increased.

**Conclusion** The result of present study indicate that the recent incidence of ALS in K area is high, the age of onset has recently become higher and the number of bulbar-onset patients has increased. All the

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