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TDP-43 is deposited in the Guam parkinsonism–dementia complex brains

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TDP-43, a nuclear factor that functions in regulating transcription and alternative splicing, was recently identified as a component of the ubiquitin-positive, tau-negative inclusions specific for frontotemporal lobar degeneration (FTLD-U) and amyotrophic lateral sclerosis (ALS). In the present study, we carried out immunohistochemical and biochemical analyses of brains of Guamanians with the parkinsonism–dementia complex (G-PDC) using anti-TDP-43, anti-tau and anti-ubiquitin antibodies. Immunohistochemistry with anti-TDP-43 antibodies revealed various types of positive structures in the frontotemporal and hippocampal regions of G-PDC cases. Most of these structures were negative for tau. By immunoblot analysis with the TDP-43 antibody, an abnormal 45 kDa band, as well as a diffuse staining throughout the gel, was detected in the sarkosyl-insoluble fractions of G-PDC brains. Dephosphorylation has shown that abnormal phosphorylation takes place in the accumulated TDP-43 seen in FTLD-U and ALS. These results suggest that accumulation of TDP-43 is a common process in certain neurodegenerative disorders, including FTLD-U, ALS and G-PDC.

Keywords: frontotemporal lobar degeneration; amyotrophic lateral sclerosis; ubiquitin; tau; inclusion

Abbreviations: ALS = amyotrophic lateral sclerosis; FTLD-U = frontotemporal lobar degeneration; G-PDC = Guam parkinsonism–dementia complex; NCI = neuronal cytoplasmic inclusions; NII = neuronal intranuclear inclusions

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Introduction

Ubiquitin-positive, tau-negative neuronal cytoplasmic inclusions (NCI) were first described in patients with amyotrophic lateral sclerosis (ALS) (Okamoto *et al.*, 1991). They were subsequently found in patients with frontotemporal lobar degeneration with motor neuron disease (FTLD-MND) (Okamoto *et al.*, 1992; Wightman *et al.*, 1992), and FTLD with MND-type inclusions but without MND (FTLD-MND-type) (Bergmann *et al.*, 1996; Jackson *et al.*, 1996; Iseki *et al.*, 1998). FTLD-MND and FTLD-MND-type are referred to as FTLD-U (Mackenzie *et al.*, 2006*a*). In some FTLD-U cases, neuronal intranuclear inclusions (NII) have been described (Woulfe *et al.*, 2001; Mackenzie and Feldman, 2003, 2005; Forman *et al.*, 2006), especially in those cases with autosomal dominant inheritance associated with mutations in progranulin gene (Baker *et al.*, 2006; Boeve *et al.*, 2006; Cruts *et al.*, 2006; Gass *et al.*, 2006; Huey *et al.*, 2006; Mackenzie *et al.*, 2006*b*; Masellis *et al.*, 2006; Mukherjee *et al.*, 2006; Pickering-Brown *et al.*, 2006; Snowden *et al.*, 2006) and in valosin-containing protein gene (Guyant-Marechal *et al.*, 2006). Recently, TDP-43, a ubiquitously expressed nuclear protein, was identified as the major component of the ubiquitin-positive inclusions in these disorders (Arai *et al.*, 2006; Neumann *et al.*, 2006, 2007; Davidson *et al.*, 2007). They include NCI, NII and dystrophic neurites in the hippocampus and frontotemporal cortex in cases of FTLD-U, and skein-like inclusions in the spinal cord in FTLD-MND and ALS cases.

The Guam parkinsonism–dementia complex (G-PDC) and amyotrophic lateral sclerosis (G-ALS) are neurodegenerative disorders of Chamorro residents of Guam. They are clinically characterized by either progressive cognitive impairment with extrapyramidal signs or motor neuron dysfunctions. G-PDC is characterized by severe neuronal loss and abundant neurofibrillary tangles (NFTs) in the temporal and frontal cortex, basal ganglia, thalamus and brainstem with a virtual absence of senile plaques (Hirano *et al*., 1961; Nakano and Hirano, 1983; Oyanagi *et al*., 1994*a*). Although environmental factors such as toxins in cycad seeds and minerals in the soils and drinking water have been implicated (Cox *et al*., 2003; Hermosura *et al*., 2005; Oyanagi *et al*., 2006), the aetiology and the pathogenesis remain unknown. G-PDC exhibits similarities to FTLD-U in terms of the frontotemporal atrophy and the occurrence of ubiquitin positive inclusions in the dentate gyrus (Oyanagi *et al*., 1994*b*; Ikemoto *et al*., 1997). In the present study, we show that various types of tau-negative, TDP-43-positive structures are present in G-PDC brains. Immunoblot analysis revealed that hyperphosphorylated TDP-43 is deposited in the sarkosyl-insoluble fractions of G-PDC brains. These results suggest that a common pathogenic mechanism through conformational changes in TDP-43 may be associated with the neurodegeneration in FTLD-U, ALS and G-PDC.

Materials and methods

Materials

Brains from six cases of clinically and neuropathologically diagnosed G-PDC, two Japanese cases with Alzheimer's disease (AD) and two non-PDC non-ALS Guamanian controls were employed in this biochemical and immunohistochemical studies. Paraffin-embedded sections from three other G-PDC cases were also used for immunohistochemistry. The age, sex, brain weight, brain regions examined and diagnosis are given in Table 1.

Immunohistochemical analysis

Small blocks of frontal regions were dissected at autopsy or from fresh frozen brain samples and fixed overnight in 10% formalin neutral buffer solution (Wako). Blocks were cut on a vibratome at 50 µm thickness. The free-floating sections were treated with 3% H_2O_2/methanol for 30 min to block the internal peroxidase and incubated in 0.5% Triton X-100/PBS for 30 min. After blocking with 10% calf serum/PBS, sections were immunostained overnight with two well-characterized antibodies to TDP-43: a polyclonal (10782-1-AP, ProteinTech Group Inc., Chicago, IL; 1 : 3000) and a monoclonal (2E2-D3, Abnova Corporation, Taipei, Taiwan; 1 : 1000). Two monoclonal antibodies to phosphorylated tau (AT8; Innogenetics, Gent, Belgium, 1 : 1000 and PHF-1; generous gift from Dr P. Davies, 1 : 2000), a polyclonal and a monoclonal antibody to ubiquitin [Z0458; Dako, Denmark; 1 : 3000 and DF2 (Mori *et al*., 1987); 1 : 200], and a monoclonal antibody to GFAP (6F2, DakoCytomation, Glostrup, Denmark; 1 : 100) were also used. For analysis of unfixed materials, Triton-X-insoluble pellets prepared from frozen brains (see below) were smeared on PLL-coated slide glasses and used. For immunostaining of the hippocampal region from G-PDC cases, 10% formalin-fixed and paraffin-embedded blocks were sectioned at 6 µm and stained with 10782-1-AP (1 : 300) and 2E2-D3 (1 : 100). Following treatment with the appropriate secondary antibody, labelling was detected using the avidin-biotinylated HRP complex (ABC) system coupled with a diaminobenzidine reaction to yield a brown precipitate. Pretreatment of tissues by autoclaving in 10 mM sodium citrate buffer for 10 min at 120°C was needed for staining with 2E2-D3 in paraffin-embedded sections.

Double-label immunofluorescence was performed using FITC and TRITC conjugated secondary antibodies. The sections were examined with a confocal laser microscope (LSM5 PASCAL; Carl Zeiss MicroImaging gmbh, Jena, Germany).

Immunoblot analysis

The sarkosyl-insoluble fractions were prepared as described (Arai *et al*., 2006) with slight modifications. Frozen temporal or frontal cortex (0.5 g) from six cases of G-PDC, two cases with AD and two controls were homogenized in 10 volumes (5 ml) of buffer A (10 mM Tris–HCl, pH 7.5 containing 1 mM EGTA, 10% sucrose and 0.8 M NaCl). After addition of another 5 ml of buffer A containing 2% Triton-X100, the homogenate was incubated for 30 min at 37°C and spun at 100 000 × *g* for 30 min at 25°C. The pellet was homogenized in 10 volume of buffer A containing 1% Sarkosyl, incubated for 30 min at 37°C and spun at 100 000 × *g* for 30 min at 25°C. The sarkosyl-insoluble pellet was homogenized in 4 volumes of buffer A containing 1% CHAPS and spun at 100 000 × *g* for 20 min.

Table 1 Description of the subjects

Case no.	Age	Sex	Brain weight (g)	Regions	Clinical diagnosis	Neuropathological diagnosis
1(CON1)	68	F	990	Frontal	Diabetes, heart failure	Normal-aged brain
2(CON2)	43	F	1370	Frontal	Burn	Slight edematous brain
3(G-PDC1)	69	F	1050	Frontal	PDC	PDC
4(G-PDC2)	69	M	875		PDC	PDC
5(G-PDC3)	52	F	1025	Frontal	PDC, myocardiac infarct	PDC
6(G-PDC4)	52	M	1025	Frontal	PDC	PDC
7(G-PDC5)	56	M	1235	Frontal	PDC, pneumonia	PDC
8(G-PDC6)	56	F	875	Frontal	PDC	PDC
9(G-PDC7)	51	F	850	Hip, temp	PDC	PDC
10(G-PDC8)	64	M	1290	Hip, Temp	PDC	PDC
11(G-PDC9)	57	F	1150	Hip, Temp	PDC	PDC
12(AD1)	82	F	670	Temp	AD	AD
13(AD2)	75	F	730	Temp	AD	AD

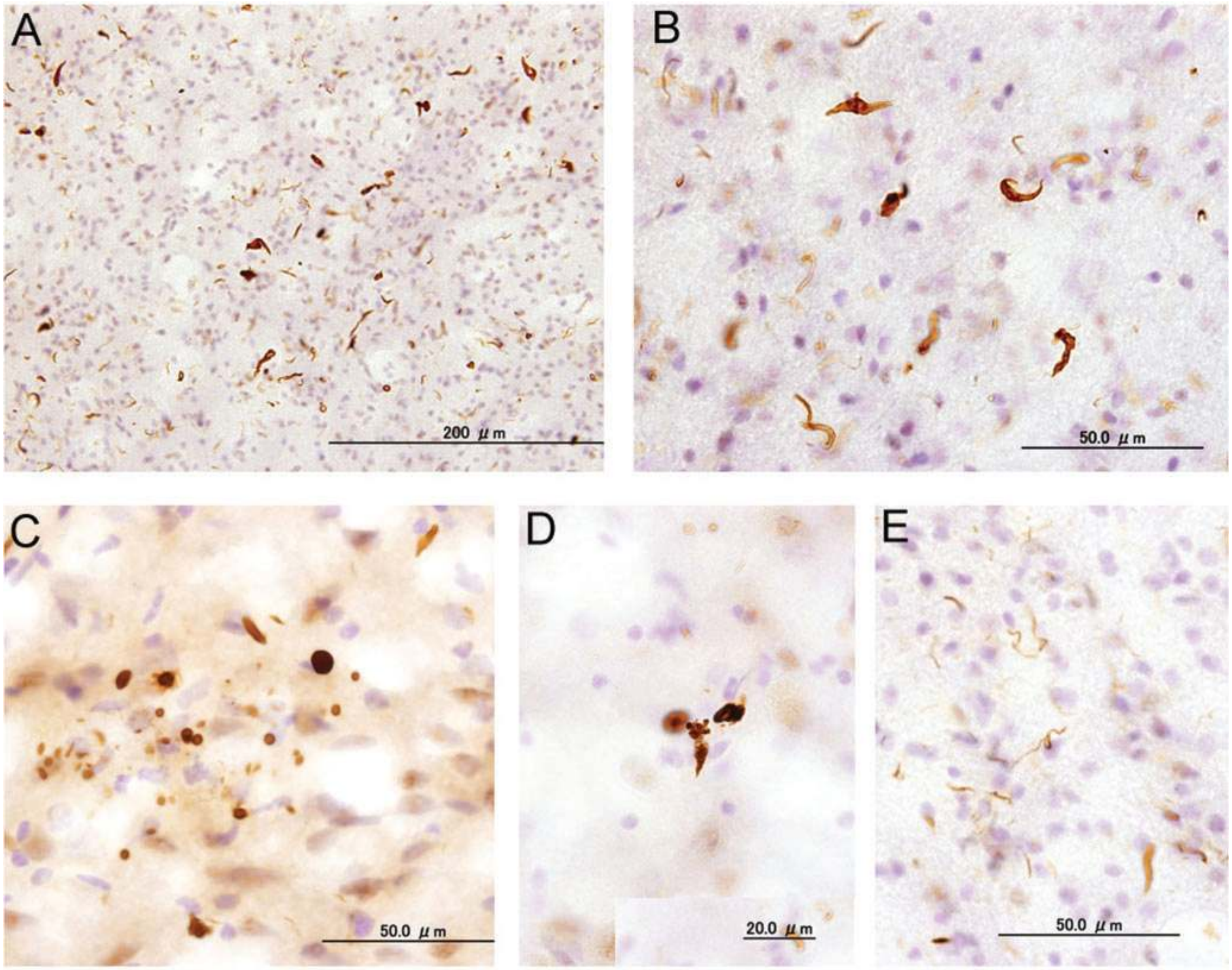

Fig. 1 Immunostainings of the frontal lobes of the G-PDC cases with the polyclonal antibody to TDP-43. (**A**) Numerous TDP-43 positive structures are observed in the white matter. (**B**) High magnification of coiled body-like or worm-like inclusions in the white matter. (**C**) Round-shape and dot-like TDP-43 positive structures in the grey matter. (**D**) Bud-shaped inclusions in the white matter. (**E**) Thin thread-like TDP-43 positive structures in the grey matter. The sections are counterstained with haematoxylin.

The pellet was sonicated in 0.8 volume of 7 M guanidine hydrochloride, dialysed against 30 mM Tris–HCl (pH 7.5), cleared by brief spin at 15 000 rpm and used for immunoblotting. For dephosphorylation, the sample was incubated with Lambda protein phosphatase as described (Arai *et al*., 2006). For the analysis of proteins in white matter and grey matter, sarkosyl-insoluble proteins before and after dephosphorylation were prepared as described (Yamazaki *et al*., 2005). Samples were run on SDS–PAGE using 10% polyacrylamide gel and the proteins were electrotransferred onto a polyvinylidene difluoride membrane, probed with the antibody to TDP-43, 10782-1-AP (1 : 3000) and the antibody to tau, HT7 (Innogenetics, Gent, Belgium; 1 : 3000), and detected as described (Arai *et al*., 2006).

Results

Immunohistochemistry of G-PDC

Immunohistochemistry of G-PDC cases with the anti-TDP-43 antibodies revealed various types of inclusions. In vibratome sections of the frontal lobe, TDP-43 positive structures with various shapes (coiled-body-like, round-shape, dot-like, bud-shaped and thin thread-like) were present in both grey matter and white matter (Fig. 1A–E). These were similarly stained with both the polyclonal and monoclonal antibodies to TDP-43. The frequency of the inclusions varied from case to case, but they were detected in all six G-PDC cases examined. The number of TDP-43 positive structures in the white matter was much greater than that of ubiquitin positive ones in each G-PDC case. No such TDP-43-positive inclusions were observed on vibratome brain sections of the AD cases and the controls.

Various types of structures were also observed in paraffin sections of the hippocampal region of G-PDC cases (Fig. 2). In the granular cells of the dentate gyrus, numerous NCI (A, B) and a few NII (C) were positive for TDP-43. The nuclear staining for TDP-43 was reduced in neurons with cytoplasmic inclusions compared to that in non-affected

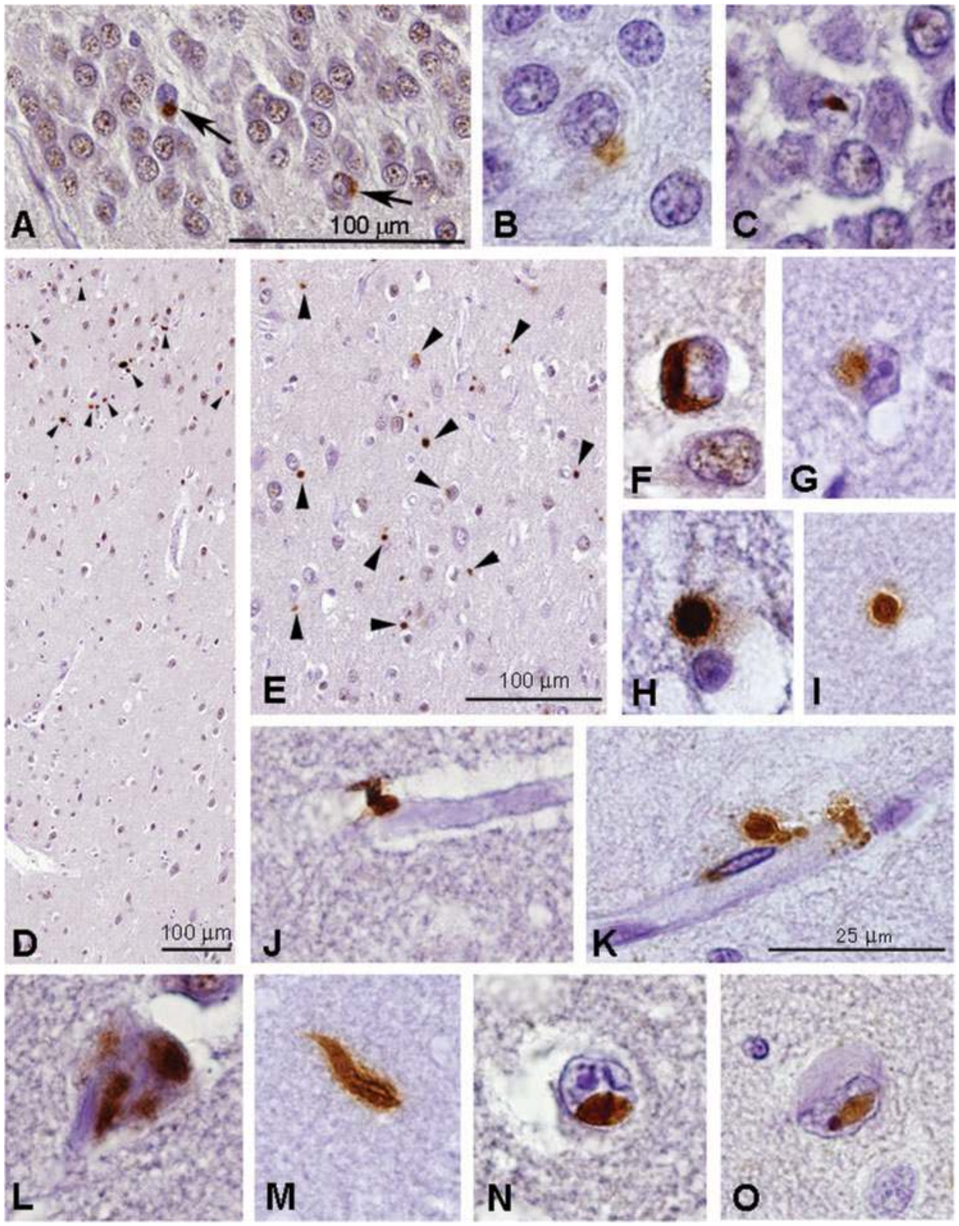

Fig. 2 Immunostainings of the hippocampus and the entorhinal cortex of the G-PDC cases. The polyclonal (**A**, **C**, **D**, **E**, **F**, **H**, **J**, **L** and **N**) and the monoclonal (**B**, **G**, **I**, **K**, **M** and **O**) antibodies to TDP-43 were used. In the granular cells of the dentate gyrus, frequent cytoplasmic inclusions with round or crescent shapes (arrows in **A**, **B**) and a few intranuclear inclusions (**C**) are observed. Note the absence of nuclear staining in neurons with cytoplasmic inclusions (arrows in **A**) compared to the granular staining of nuclei in nonaffected neurons. In the superficial layer of the entorhinal cortex, robust TDP-43 positive structures are seen (arrowheads in **D**, **E**). They include neuronal cytoplasmic inclusions (**F**, **G**), round structures (**H**, **I**), those associated with small blood vessels (**J**, **K**) or NFTs (**L**), the thread-like structure (**M**) and the intranuclear inclusions (**N**, **O**). The sections are counterstained with haematoxylin. Except for **A**, **D** and **E**, the same magnification is used as shown in **K**.

neurons as previously reported (Neumann *et al*., 2006; Davidson *et al*., 2007). In the parahippocampal cortex, TDP-43 positive structures tended to be more abundant in the superficial layer than in the deep layer (D, E). In addition to NCI (F, G), numerous round structures with similar size to glial nuclei were seen (H, I). There were also structures with round, dot-like or granular shape associated with small vessels (J, K). Occasional immunopositive structures associated with NFT were observed (L). Thread-like structures (M) and NII (N, O) were occasionally seen. All of these structures were positive for both the polyclonal and monoclonal antibodies to TDP-43, although the immunoreactivity was stronger with the polyclonal than with the monoclonal.

Nuclear TDP-43 staining varied much from case to case even in controls as previously reported (Davidson *et al*., 2007). Furthermore, within cases showing TDP-43 nuclear staining, this was not evenly present in all nuclei, namely, a

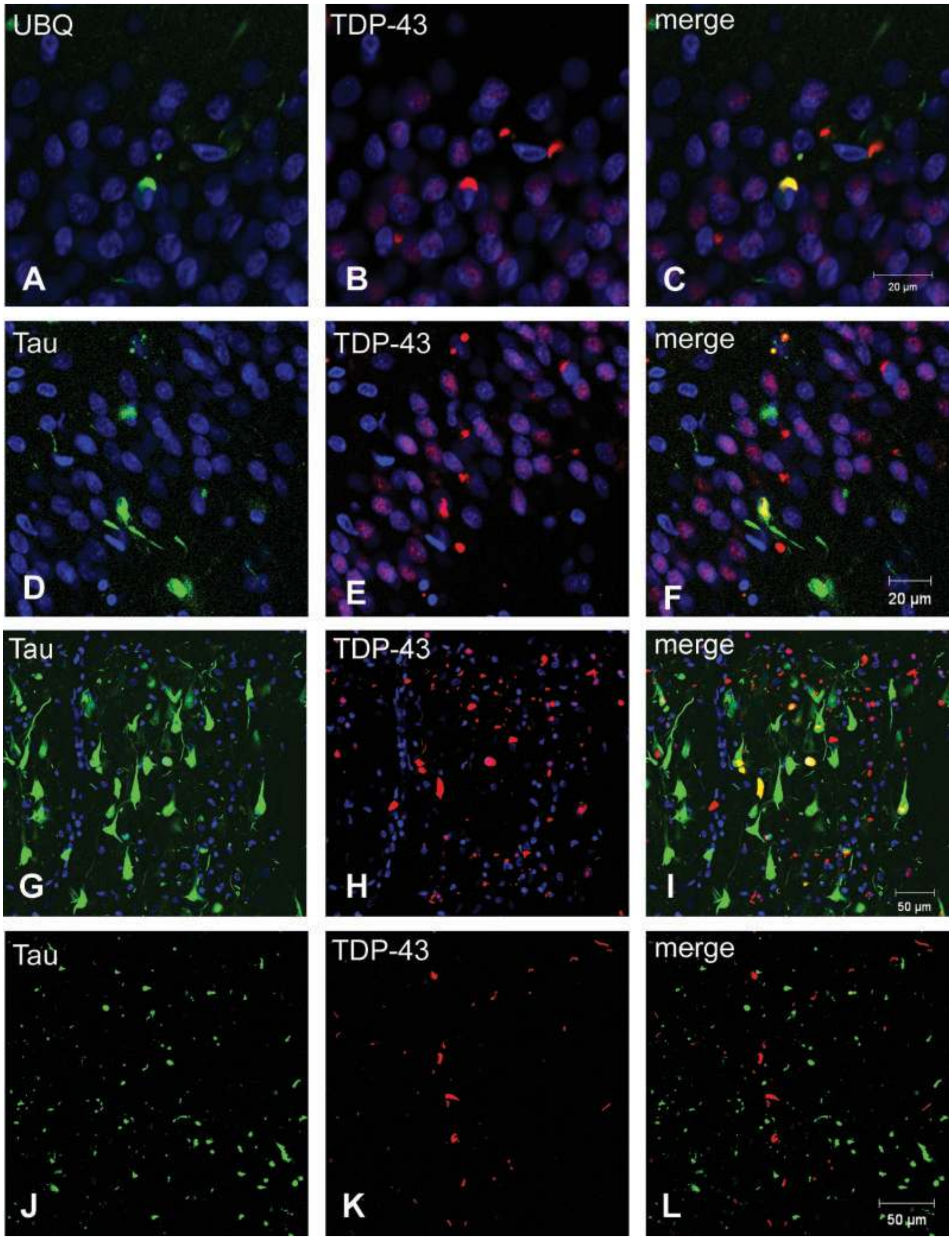

Fig. 3 Double-label immunofluorescence of the G-PDC cases with anti-ubiquitin (DF2 in **A**) or anti-tau (PHF-1 in **D** and **G**; AT8 in **J**) and anti-TDP-43 (**B**, **E**, **H** and **K**). Merged images are shown in **C**, **F**, **I** and **L**. In the granular cells in the dentate gyrus, a crescent inclusion shows colocalization (yellow fluorescence in **C**) of ubiquitin (green fluorescence in **A**) and TDP-43 (red fluorescence in **B**). Structures which are ubiquitin positive and TDP-43 negative (green in **C**) or ubiquitin negative and TDP-43 positive (red in **C**) are also observed. In the same region, colocalization of tau and TDP-43 are seen in some structures (yellow in **F**), but many TDP-43 positive structures are negative for tau (red in **F**). In the temporal cortex (**G**–**I**), most of the structures stained for tau (**G**) and those stained for TDP-43 (**H**) are independent, although colocalization of tau and TDP-43 is observed in part of NFTs (**I**). In the white matter of the frontal lobe, the distribution of structures positive for tau (**J**) and of those positive for TDP-43 (**K**) are virtually independent (**L**). In **A**–**I**, the cell nuclei are stained with TO-PRO-3 (Invitrogen, Tokyo, Japan), producing a blue colour. Scale bars are shown in **C**, **F**, **I** and **L**.

mix of TDP-43 positive and negative nuclei was seen (data not shown).

Figure 3 shows double immunofluorescence staining with anti-ubiquitin (A) or anti-tau (D, G and J) and anti-TDP-43 antibodies (B, E, H and K). Some NCI in the dentate gyrus were positive for both ubiquitin and TDP-43 (C). Most TDP-43 positive structures in the dentate gyrus and the temporal cortex were negative for tau, although

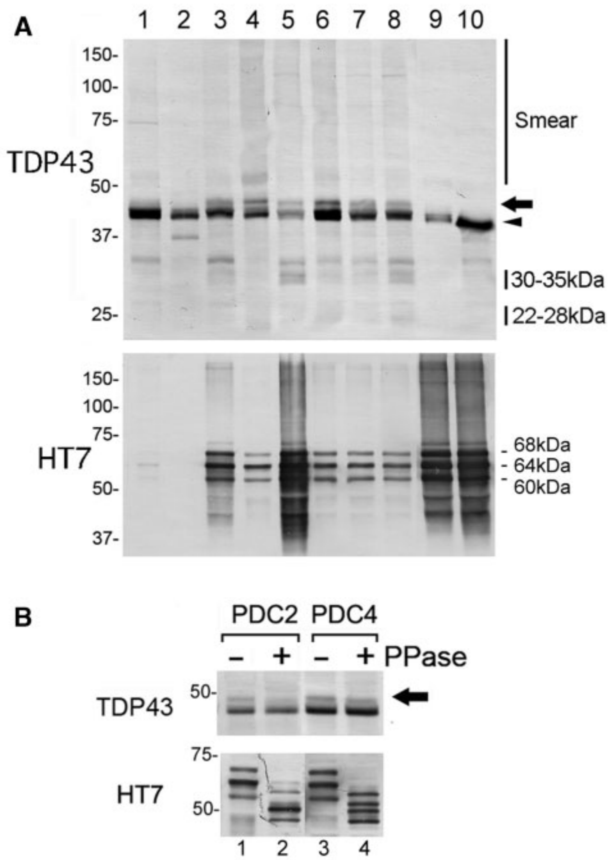

Fig. 4 Immunoblotting of the sarkosyl-insoluble fraction with anti-TDP-43 antibody (upper panel) and HT7 (lower panel). (**A**) Lanes 1 and 2, control (cases 1 and 2); lanes 3–8, G-PDC (cases 3–8); lanes 9 and 10, AD (cases 12 and 13). A positive band of 43 kDa (arrow head) is commonly seen in all cases, while an additional band of 45 kDa (arrow) as well as diffuse smear stainings are observed only in G-PDC brains (lanes 3–8). Several bands with 30–35 or 22–28 kDa are evident in five of six G-PDC cases (lanes 3, 5–8), although weak bands at 30–35 kDa are visible in a control case (lane 1) and in an AD case (lane 10). The triplet bands of 68, 64 and 60 kDa, typical for hyperphosphorylated tau in AD, and additional smear immunoreactivities are detected in all G-PDC cases examined. However, the intensities are much weaker than those found in two typical AD cases. (**B**) Immunoblotting of sarkosyl-insoluble fractions from the G-PDC cases before and after dephosphorylation. Partial mobility shift of the 45 kDa TDP-43-positive band (arrow) is observed after dephosphorylation of samples with lambda protein phosphatase. Mobility shifts in the PHF-tau bands are also detected with the HT7 antibody.

parts of NFTs were positive for TDP-43 (F, I). Virtually, no colocalization of tau and TDP-43 was observed in the white matter of the frontal lobe (L).

Immunoblot analysis of G-PDC

Figure 4 illustrates the results of immunoblotting of sarkosyl-insoluble fractions from two controls, six G-PDC cases and two AD cases with an anti-TDP-43 antibody or a phosphorylation independent anti-tau antibody HT7. By immunoblotting with HT7, the three major abnormal tau bands of 60, 64 and 68 kDa were detected in all G-PDC cases (Fig. 4A, lower panel). Although the pattern was indistinguishable to that seen in AD brains, the intensities of these tau bands in G-PDC cases were apparently weaker than those in two AD cases. By immunoblotting with the anti-TDP-43 antibody, a major band of 43 kDa corresponding to full-length TDP-43 was seen in all samples examined. In addition to the 43 kDa band, an abnormal 45 kDa band was observed in all G-PDC cases examined (lanes 3–8) which was not seen in the two controls (lanes 1 and 2) and two AD cases (lanes 9 and 10) (Fig. 4A, upper panel). Moreover, a diffuse smear staining was more prominent in G-PDC cases than in controls and AD cases (Fig. 4A, upper panel). Several positive bands of 30–35 or 22–26 kDa were evident in five of six G-PDC cases (lanes 3, 5–8), although faint bands at 30–35 kDa were visible in a control case (lane 1) and in an AD case (lane 10). After dephosphorylation of the samples with lambda protein phosphatase, a partial shift of the 45 kDa band was observed (Fig. 4B), suggesting that phosphorylation takes place in the full-length TDP-43. Similar results were obtained in the experiments with alkaline phosphatase at 37°C for 2 h (data not shown).

In order to confirm the deposition of TDP-43 in the white matter biochemically, the grey and white matters of two G-PDC cases were separated from each other macroscopically and the sarkosyl-insoluble fractions were immunoblotted with the anti-TDP-43 antibody. The abnormal 45 kDa band and smear stainings were detected in both the grey matter and the white matter (Fig. 5) and the intensities were slightly stronger in the sarkosyl-insoluble fractions from the white matter than in those from the grey matter in both cases (Fig. 5, upper panel). In contrast, immunoreactivities of tau bands detected with HT7 were much stronger in the grey matter than in the white matter in case PDC1 and similar deposits were detected in case PDC5 (Fig. 5, lower panel).

Discussion

TDP-43 is thought to function in transcriptional repression and exon skipping (Buratti *et al.*, 2001; Wang *et al.*, 2002; Ayala *et al.*, 2005; Buratti *et al.*, 2005). It was first identified as a protein capable of binding to a TAR DNA of the human immunodeficiency virus 1 (HIV-1) long terminal repeat region (Ou *et al.*, 1995). TDP-43 interacts with several nuclear ribonucleoproteins including heterogeneous nuclear RNP A/B and survival motor neuron protein, inhibiting alternative splicing (Wang *et al.*, 2002; Buratti *et al.*, 2005). The physiological function of TDP-43 in brain cells has not yet been determined. The present study showed numerous TDP-43 positive, tau-negative structures with various types of morphologies in white and grey matters of G-PDC brains.

Ubiquitin-positive inclusions have already been described in the granular cells of the hippocampal dentate gyrus in G-PDC cases. Most of these ubiquitin-positive inclusions

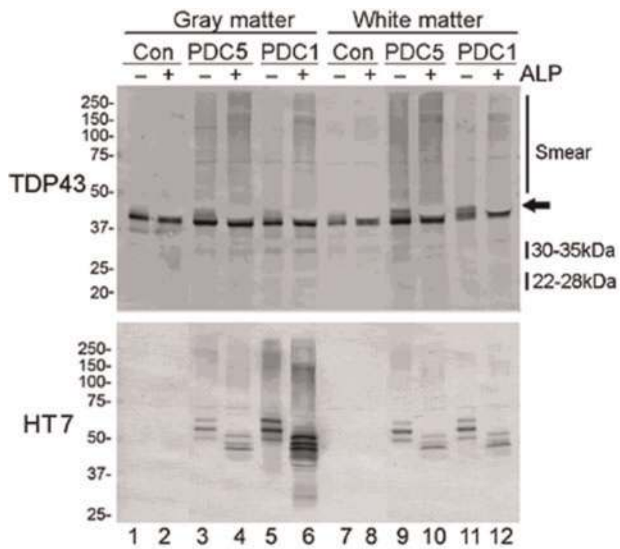

Fig. 5 Immunoblotting of the sarkosyl-insoluble fractions from grey or white matters of G-PDC cases with anti-TDP-43 antibody and HT7 before and after dephosphorylation. Lanes 1, 2, 7 and 8, control (case 2); lanes 3, 4, 9 and 10, G-PDC5 (case 7); lanes 5, 6, 11 and 12, G-PDC1 (case 3). Sarkosyl-insoluble fractions from grey matter (lanes 1–6) and white matter (lanes 7–12) before (lanes 1, 3, 5, 7, 9 and 11) and after (lanes 2, 4, 6, 8, 10 and 12) treatment with alkaline phosphatase (ALP) are immunoblotted with the TDP-43 antibody (upper panel) or HT7 (lower panel). The 45 kDa TDP-43 band (arrow) and the smeared stainings, which are not seen in control cases, are detected in both grey and white matter of the G-PDC cases. In both G-PDC cases, the intensities of the immunoreactivities for TDP-43 are stronger in the white matter than in the grey matter. In contrast, abundant tau is detected in the grey matter of the G-PDC1 case but less tau is observed in the white matter of G-PDC1 and in grey and white matter of G-PDC5 cases.

were reported to be tau-positive (Ikemoto *et al.*, 1997). In the present study, however, we showed that most of the TDP-43 positive inclusions in the granular cells in the hippocampus of G-PDC cases were negative for tau. These findings suggest that the occurrence of TDP-43 positive and tau negative neuronal inclusions in the hippocampus is common to FTLD-U, ALS and G-PDC. Some of the TDP-43-positive inclusions were also immunoreactive for ubiquitin, suggesting that partial ubiquitination may occur on the deposited TDP-43, as seen on tau in AD or α-synuclein in DLB.

On the other hand, the morphology and the distribution of some TDP-43 positive structures in the G-PDC cases seem to be different from those reported in FTLD-U cases (Arai *et al.*, 2006; Neumann *et al.*, 2006; Davidson *et al.*, 2007). For instances, in the frontal region, TDP-43 positive structures with various shapes were more pronounced in the white matter than in the grey matter in G-PDC cases, whereas NCI and dystrophic neurites were prominent in the superficial cortical layer in FTLD-U cases. The TDP-43 positive round structures (Fig. 2H and I) and those associated with small vessels (Fig. 2J and K) found in the parahippocampal and temporal cortices of G-PDC cases in this study have not so far been described in FTLD-U cases. These structures might not be considered corpora amylacea, based on the following points. First, the double immunofluorescence staining with antibodies to GFAP and TDP-43 showed that these TDP-43 positive structures were negative for GFAP (data not shown). Second, the laminar distribution of those was different from that of corpora amylacea, which is reported to be common in the surface glial feltwork in the outer part of layer I covering the cortex (Cavanagh, 1999). Finally, pretreatment of the section with 1 N KOH, which is reported to reduce the staining of corpora amylacea (Cavanagh, 1999), did not affect the staining of these structures with anti-TDP-43 antibodies (data not shown). It also seems unlikely that these TDP-43 positive round structures are normal nuclei since these are negative for haematoxylin (Fig. 2H–K) and for TO-PRO-3 (Fig. 3H). We speculate the possibility that these are degenerating nuclei or swollen processes like spheroids, but the nature of those should be further investigated.

The present biochemical studies demonstrate that hyperphosphorylated TDP-43 with a molecular weight of 45 kDa, fragments or splicing isoforms with lower molecular weight and the smearing substances with diffuse staining, similar to those found in FTLD-U and ALS, were deposited in the sarkosyl-insoluble fractions of G-PDC brains. The recovery of normal full-length TDP-43 in the sarkosyl-insoluble fraction might be due to its presence in the nucleus. These results suggest that accumulation of TDP-43 is a common process in certain neurodegenerative disorders, including ALS, FTLD-U and G-PDC, and similar biochemical alterations and conformational changes in TDP-43 may occur in these diseases.

It is unclear whether there are any relationships between the deposition of hyperphosphorylated tau and the accumulation of TDP-43. The occasional occurrence of TDP-43-positive structures associated with NFTs in the hippocampal and temporal regions of G-PDC cases may indicate some association between tau and TDP-43. However, it has to be noted that in a case of G-PDC (PDC3), the western blot of the sarkosyl insoluble fraction showed the most abundant tau (Fig. 4A, lower panel, lane 5) but the least amount of TDP-43 (Fig. 4A, upper panel, lane 5) among the all PDC cases examined. Although some unique tau positive structures, such as the granular hazy inclusions in astrocytes and the fine granules in white matter, have been previously reported in G-PDC (Oyanagi *et al.*, 1997; Yamazaki *et al.*, 2005), the association between these structures and TDP-43 was not examined in this study. Further studies will be needed to elucidate the role of the association between tau and TDP-43 in the pathogenesis of G-PDC.

There has been a long history of debate for the nosology of G-PDC. It is distinguished from AD by the laminar distribution of NFT (Hof *et al.*, 1991), the prominent glial pathology (Oyanagi *et al.*, 1997) and the relative absence of amyloid plaques (Gentleman *et al.*, 1991; Schmidt *et al.*, 1998). The nature of α-synuclein pathology is also different between Parkinson's disease (PD) and G-PDC, i.e. the frequency of Lewy bodies in the substantia nigra is lower in G-PDC than in PD (Hirano *et al.*, 1966; Oyanagi and Wada, 1999), while the density of α-synuclein positive structures in the cerebellum is higher in G-PDC than in PD (Sebeo *et al.*, 2004). As for TDP-43, the predominance of white matter TDP-43 profiles is very unlike FTLD-U variants so far described. These findings suggest that G-PDC represents combined neurodegenerative disorders, in which tau, α-synuclein and TDP-43 are simultaneously involved, but does not represent mere co-existence of multiple common degenerative diseases, including AD, PD and FTLD-U.

In conclusion, the results of the present study suggest that a common pathogenic mechanism through the process of biochemical and structural changes in the TDP-43 molecule in neurons and/or glial cells may be related to the neurodegeneration in ALS, FTLD-U and G-PDC. The deposition of TDP-43 in brains of G-ALS patients should be analysed as well. It might also be important to investigate the relationship between environmental or genetic factors and dysfunction or deposition of TDP-43 in these disorders.

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