Fig. 2. Brain SPECT showed hypoperfusion in the bilateral thalamus with a left predominance as well as the medial frontal lobe cortices (A). Images of eZIS demonstrated regions showing a Z-score of 2 or more in the medial frontal lobe cortices and thalamus (B).

of PSP-RS in the early stage of the disease. In this patient, images of eZIS were useful to clearly demonstrate the brain regions showing hypoperfusion.

Recent studies using functional brain images have shown the involvement of the frontal lobe cortex and thalamus in patients with probable PSP-RS [9–11]. Because imbalance and falls as initial manifestations of most cases of PSP-RS are closely associated with thalamic dysfunction [22], thalamus may be involved from the early stage of PSP-RS. Intriguingly, thalamic involvement in patients with PSP-RS is considered to be a consequence of disrupted cholinergic fibers ascending from brainstem nuclei such as the pedunculopontine nuclei to thalamus rather than the impairment of cholinoceptive neurons in the thalamus [23]. This pathomechanism may explain the fact that thalamic degeneration is not necessarily severe in brains of autopsied patients with PSP-RS.

In neurodegenerative diseases, thalamic involvement on functional brain images has been reported not only in PSP-RS but also in the thalamic form of the MM2 subtype of sCJD [15], although the latter is extremely rare when compared to the former. Importantly, a proportion

of patients with this type of sCJD present with clinical features of PSP-RS [15,16]. Because characteristic EEG and MRI abnormalities are almost absent in patients with this type of sCJD [15,16], a rapidly progressive course of PSP-RS may need the suspicion of an underlying sCJD pathology regardless of the absence of supportive findings on EEG or MRI [16].

So far, functional brain image findings have hardly been reported in patients with definite PSP-RS. While the study by Kimura et al. included one patient with definite PSP-RS, details of the clinical course of this patient are not described [11]. Thus, this is the first report of a case of definite PSP-RS in which thalamic involvement was demonstrated in the early stage of the disease. In the future, a large number of patients with PSP-RS should be studied employing functional brain images and autopsy to clarify whether thalamic involvement is a constant finding in the early stage of definite PSP-RS.

Conflict of interest statement
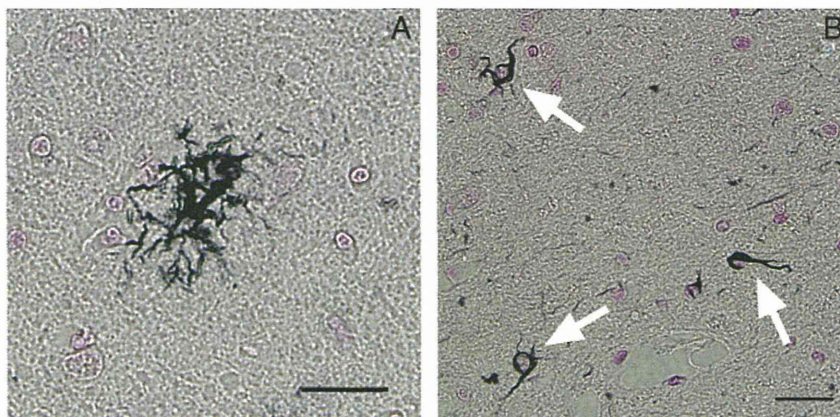
The authors have no conflicts of interest.

Fig. 3. Gallyas staining demonstrated a tuft-shaped astrocyte (A) and coiled bodies (B, arrows) in the frontal lobe cortices. Scale bars = 20 μm.

Acknowledgment

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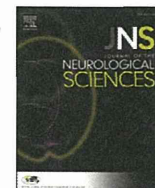
Pathological features of FTLD-FUS in a Japanese population: Analyses of nine cases

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ABSTRACT

We investigated the pathological features of frontotemporal lobar degeneration (FTLD) with fused in sarcoma protein (FUS) accumulation (FTLD-FUS) in the Japanese population. Only one out of nine FTLD-FUS cases showed pathology that corresponds to atypical FTLD with ubiquitin-positive inclusions (aFTLD-U). Five were basophilic inclusion body disease (BIBD) and two were neuronal intermediate filament inclusion disease. The last case was unclassifiable and was associated with dystrophic neurites (DNs) as the predominant FUS pathology. The results of this study indicate an ethnic difference from western countries. In Japan, BIBD is the most common subtype of FTLD-FUS and aFTLD-U is rare, a finding which contrasts with aFTLD-U being the most common form in western countries. Immunohistochemical analyses of these FTLD-FUS cases reveal that FUS abnormally accumulated in neuronal cytoplasmic inclusions (NCIs) and DNs has an immunohistochemical profile distinct from that of normal, nuclear FUS. NCIs and DNs are more readily stained than the nuclei by antibodies to the middle portion of FUS. Antibodies to the carboxyl terminal portion, on the other hand, stain the nuclei more readily than NCIs and DNs. Such an immunohistochemical profile of NCIs and DNs was similar to that of cytoplasmic granular FUS staining which we previously reported to be associated with dendrites and synapses. Redistribution of FUS from the nucleus to the cytoplasm could be associated with the formation of abnormal FUS aggregates in FTLD-FUS.

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1. Introduction

Following the identification of mutations in the gene encoding the fused in sarcoma protein (FUS) as the cause of familial amyotrophic lateral sclerosis (FALS) type 6, where FUS was shown to be accumulated in motor neurons [1,2], deposition of FUS in inclusions was discovered in frontotemporal lobar degeneration (FTLD) [3] in which the inclusions are negative for tau and TAR DNA-binding protein 43 (TDP-43). A large part of FTLD with ubiquitin-positive inclusions (FTLD-U), i.e., tau negative FTLD, is FTLD with TDP-43 accumulation (FTLD-TDP), but 5–20% of FTLD-U cases are TDP-43 negative. The vast majority of such cases are now considered to be FTLD with FUS accumulation (FTLD-FUS) [4–7]. At present, neither abnormal phosphorylation nor truncation has been shown in accumulated FUS, an observation which contrasts with other abnormally accumulated proteins, such as tau, α-synuclein and TDP-43, in neurodegenerative diseases. The only reported evidence for

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a biochemical change of abnormally accumulated FUS is increased insolubility [3].

FTLD-FUS is considered to consist of three pathological subtypes, atypical FTLD-U (aFTLD-U), basophilic inclusion body disease (BIBD) and neuronal intermediate filament inclusion disease (NIFID) [3,5,8–12]. Of these three subtypes, aFTLD-U is characterized neuropathologically by the occurrence of neuronal cytoplasmic inclusions (NCIs) positive for ubiquitin but mostly invisible by hematoxylin and eosin (HE) staining. In western countries, aFTLD-U is the most common subtype of FTLD-FUS [12–14]. NIFID is characterized by NCIs that contain class IV neuronal intermediate filaments [15–18]. NCIs in this subtype sometimes have HE-unstained central compartments [15] or eosinophilic cores [11,15,17,19]. In BIBD, some, but not all, NCIs are recognized as lightly basophilic inclusions by HE staining. While these three subtypes may represent a continuous spectrum of diseases [10], recent detailed histopathological comparison has suggested that they are closely related but distinct entities [5]. Basophilic inclusions are also found in FALS type 6, but BIBD as a subtype of FTLD-FUS is now clearly distinguished in that it is a sporadic disease without FUS gene mutations and the inclusions are, similarly to those in aFTLD-U and NIFID, positive for other members of the FET protein family, Ewing's sarcoma and TATA-binding protein-associated factor 15 [20,21].

In Japan, a number of differences from western countries have been recognized in the occurrence of diseases in the FTLD categories. While the majority of FTLD in western countries shows frontotemporal dementia (FTD), which is often referred to as a behavioral variant of FTD (bvFTD), semantic dementia accounts for a significant portion of FTLD in Japan [22]. From a genetic point of view, the incidence of familial FTLD is much lower in Japan than those reported in western countries [23]. Further, most familial FTLD in Japan is associated with tau mutations and FTLD with granulin mutations or C9orf72 repeat expansion is rare [24]. Considering these ethnic differences, we have reviewed all FTLD cases archived in the brain collection in the Tokyo Institute of Psychiatry, including some cases shared under collaboration with other laboratories and other cases autopsied in the Tokyo Metropolitan Matsuzawa hospital. Of 66 FTLD cases, we have found nine FTLD-FUS cases, of which only one shows pathology consistent with aFTLD-U. We have also found a characteristic immunohistochemical profile of abnormally accumulated FUS using a panel of anti-FUS antibodies that recognize different portions of the FUS molecule.

2. Materials and methods

2.1. Neuropathological examination

Brain and spinal cord tissue samples were fixed postmortem with 10% formalin and embedded in paraffin. Ten micrometer thick sections were prepared from the cerebrum, midbrain, pons, medulla oblongata, cerebellum and spinal cord. Sections were examined initially with HE, Klüver–Barrera (KB), Bodian's silver, methenamine silver and Gallyas–Blaak staining. Severity of the central nervous system (CNS) lesions was evaluated semiquantitatively using the grading system employed in a previous study [19].

2.2. Immunohistochemical staining

For immunostaining, deparaffinized sections were incubated with 1% H_2O_2 in methanol for 30 min to eliminate endogenous peroxidase activity in the tissue. Sections were pretreated by autoclaving for 10 min in 10 mM sodium citrate buffer, pH 6.0, at 120 °C. After washing three times with 0.01 M phosphate buffered saline (PBS), pH 7.4, sections were blocked with 10% normal serum from the appropriate animal species. Sections were then incubated overnight at 4 °C with one of the primary antibodies in PBS containing 0.3% Triton X-100 (PBS-Tx). After washing three times in PBS-Tx, sections were incubated in biotinylated

anti-mouse or anti-rabbit secondary antibody for 1 h, and then in avidin-biotinylated horseradish peroxidase complex (ABC Elite kit, Vector) for 1 h. Peroxidase labeling was visualized with 0.2% 3,3'-diaminobenzidine (DAB) as a chromogen. Sections were counterstained with hematoxylin.

The antibodies employed for immunohistochemistry are listed in Table 1. For α-internexin staining, dilution of the antibody was chosen for each case so that we obtained weak axonal and neuronal cytoplasmic staining as an internal positive control. α-Internexin staining in 6 out of 9 cases was published previously with a different antibody (ab32306, rabbit, polyclonal, 1:100, Abcam plc) [19], and the results were consistent between the two studies.

As reported previously [3,11], sensitivity of FUS immunostaining varied among cases, presumably because of the diverse fixation conditions. Therefore, the dilution of the primary antibody was adjusted to obtain FUS immunoreactivity of neuronal nuclei in each case. Sometimes, positive staining of NCIs but not nuclei, or vice versa, was obtained even at the highest concentration of the antibody, where the non-specific background was about to blur specific labeling. To overcome such uncertainty, we used 6 anti-FUS antibodies raised against 5 different portions of the FUS molecule (Table 1).

2.3. Cases

From 1973 to 2011, the period between the foundation and closure of the Tokyo Institute of Psychiatry, 66 FTLD cases were registered in the neuropathology department of the institute. All cases fulfilled the clinical and pathological diagnostic criteria for FTLD. Of the 66 cases, 9 were FTLD-FUS. Distinctions between FTLD-FUS subtypes are based on the documentation in the literature [3,5,6,10–12]. Briefly, the diagnosis of BIBD was made when a significant number of basophilic or lightly-basophilic NCIs were found in HE staining. It is sometimes difficult to distinguish the NCIs in BIBD from those in NIFID in HE-stained sections [19]. Thus, the diagnosis of NIFID eventually relied on the result of α-internexin immunohistochemistry, where a significant number of NCIs in the neocortex were positive for α-internexin in NIFID cases. Occasional neuronal intranuclear inclusions (NIIs) also suggested that a case was unlikely to be BIBD [5,9,10]. In case 8, most NCIs were invisible in HE-stained sections and were negative for α-internexin. Together with relatively frequent NIIs in the hippocampus compared with other cases in our series, we considered this case to be aFTLD-U.

3. Results

The basic clinical information and pathological subtypes of the 9 FTLD-FUS cases are summarized in Table 2. No case had a family history of similar disorders or amyotrophic lateral sclerosis. The mean age at

Table 1
Antibodies used for immunohistochemistry.

Antibody	Type	Company	Concentration[a]
Anti-ubiquitin	Rabbit polyclonal	Dako	0.77 μg/ml
Anti-TDP43	Rabbit polyclonal	Proteintech Group	0.53 μg/ml
Anti-α-internexin (2E3)	Mouse monoclonal	Santa Cruz Biotechnology	1:100
Anti-FUS			
FUS [1–50]	Rabbit polyclonal	Abcam	1:10 to 1:100
FUS [1–50]	Rabbit polyclonal	Bethyl Laboratories	3.3–10 μg/ml
FUS [52–400]	Rabbit polyclonal	Proteintech Group	13 μg/ml
FUS [86–213]	Rabbit polyclonal	Sigma-Aldrich	0.63–6.3 μg/ml
FUS [250–300]	Rabbit polyclonal	Abcam	10–50 μg/ml
FUS [400–450]	Rabbit polyclonal	Bethyl Laboratories	50 μg/ml
FUS [500–526]	Rabbit polyclonal	Bethyl Laboratories	10–100 μg/ml

[a] When the concentration of antibody was not available in the data sheet, dilution from the original solution provided by the distributor was shown. The numbers in [] indicate amino acid sequences of the antigen to which the antibodies were raised.

onset was 44 years old and the average duration of illness was 8.5 years. Initial symptoms and signs in 7 cases were of bvFTD, which is consistent with reports from western countries [3,10,11]. It has to be noted that cases 2 and 3 were included in two of the previous studies with FUS immunohistochemistry [5,10], and case 9, a case with unusual FUS pathology, has been reported elsewhere [25].

The details of the clinical and pathological features, except for the FUS immunopathology, were reported previously in cases 1–7 and 9 [19,26–33]. In summary, the brain weights ranged from 880 to 1230 g. The distribution of cerebral atrophy varied from case to case, although it was confined to either the frontal or the temporal cortex or both (Table 3). All cases showed severe atrophy of the caudate head. The detailed distribution and severity of degeneration in cases 1–4, 6, 7 and 9 have been described in previous reports [19,25], so we have added the corresponding data for cases 5 and 8 and assembled them into supplementary Fig. 1. Of the newly added cases, case 5 was fully compatible with BIBD and case 8 appeared to be aFTLD-U, although the number of NII was modest. Various numbers of round or oval-shaped NCIs were found with HE (Fig. 1A), KB and Bodian's silver stainings in the affected CNS regions in cases 1 through 7. The broad distribution of NCIs in the 4 BIBD cases, cases 1 through 4, and 2 NIFID cases was reported previously [5,10,19,31]. In case 5, NCIs were present similarly to other BIBD cases in the frontal and temporal cortices, hippocampus, amygdala, basal ganglia and brain stem nuclei such as the pontine nucleus. In an NIFID case, case 6, a few NCIs in the hippocampus contained eosinophilic cores (Fig. 1B) [11,15,17,19] and, in the other NIFID case, case 7, some NCIs had faintly stained compartments outlined with relatively intense staining with HE and KB (Fig. 1C) [15]. In cases 8 and 9, no NCIs were discerned with HE and KB staining.

Ubiquitin immunohistochemistry revealed a small number of NCIs in cases 5, 6, 8 and 9 but not in other cases. In case 8, only occasional ubiquitin-positive vermiform NIIs were seen and limited to the hippocampal dentate granular cells. In the two NIFID cases, many NCIs were positive for α-internexin (Fig. 1D). α-Internexin positive NCIs were also present, though rare, in cases 1 and 4. In the other cases, no apparent α-internexin positive inclusions were found, but diffuse and intense neuronal cytoplasmic staining was seen frequently in degenerated brain regions. Such intense cytoplasmic staining was often difficult to distinguish from crescent and annular NCIs.

FUS immunohistochemistry showed numerous NCIs and a few DNs in the frontal and temporal cortices in cases 1 through 8. The NCIs bearing eosinophilic core in case 6 were positive for FUS, but the cores themselves were either negative or only weakly positive for FUS (Fig. 1E)

Table 3
Findings of FUS immunohistochemistry using multiple antibodies.

Case #		1	2	3	4	5	6	7	8	9
Epitope	1–50 (Bethyl)	I	I	I	I	N	N	N	I	N
	1–50 (Abcam)	I	I	I	I	U	N	N	I	I
	52–400	I	I	I	I	I	I	I	I	I
	86–213	I	I	I	I	I	I	I	I	I
	250–300	–	I	–	–	–	I	U	–	–
	400–450	U	U	N	N	N	N	N	N	N
	500–526	–	–	N	N	N	N	N	N	–

I: inclusions are stained more readily than the nuclei; N: the nuclei are stained more readily than inclusions; U: the difference between the nuclei and inclusions is unclear; –: no positive staining is obtained.

Fig. 1F illustrates that the NCIs visible on HE-stained sections are variably immunoreactive for FUS. In cases 1 through 7, the size and morphology of NCIs were variable, being annular, crescent, tangle-like, oval or round (Fig. 1G), while, in case 8, small, round NCIs predominated in the cerebral cortex (Fig. 1H). GCIs were found only in cases 1, 6 and 7. Sparse NIIs were seen in the hippocampal CA1, transentorhinal cortex, globus pallidus and putamen in case 7 (Fig. 1I) and in the hippocampal dentate granular cells in case 8 (Fig. 1J). Case 9 was atypical in that DNs predominated over NCIs in the cerebral cortex (Fig. 1K) and that, in addition to FUS accumulation in the brain, TDP-43 positive NCIs were present in the brain stem and the spinal cord. TDP-43 positive inclusions were not seen in the other 8 cases. The details of case 9 were reported elsewhere [25,30]. The FUS pathology of this case did not correspond to any known subtype. In summary, FTLD-FUS subtypes in this archive are as follows: 5 BIBD, 2 NIFID, 1 aFTLD-U and 1 unclassifiable.

Because of the inconstant staining intensity of FUS immunohistochemistry among cases, we applied multiple antibodies that were raised against various portions of the FUS molecule to the neocortex and hippocampus sections of all cases. The results of FUS immunostaining with these antibodies were variable, depending on the localization of antigen epitopes, and could be divided into two patterns: type I in which NCIs and DNs were more intensely or readily stained than the neuronal nuclei, and type N in which nuclei were stained better than the inclusions (Figs. 2 and 3). If the difference in staining intensity was not easily evident, the patterns were determined by staining the sections at different concentrations of the antibody. In type I, NCIs were stained positively at a lower concentration than

Table 2
Clinical and pathological features.

Case no./sex	Age at onset, year	Disease duration, year	Initial symptoms	Prominent features	Cerebral regions showing severe atrophy	Pathological subtype	Reference
1/M	34	6.3	Weakness in the left hand, dysarthria	Lower motor neuron signs	Temporal pole	BIBD	[19,26,31]
2/M	57	6	Obsessive behaviors	Behavioral abnormality	Frontal pole, temporal pole, temporal base	BIBD	[5,10,19,27,31]
3/F	56	12	Behavioral abnormality, memory impairment, altered eating habit	Behavioral abnormality	Frontal pole, temporal pole temporal base	BIBD	[5,10,19,28,31]
4/M	40	7	Disinhibition	Behavioral abnormality	Frontal base temporal base	BIBD	[19]
5/M	44	3.3	Overeating	Behavioral abnormality	Anterior portion of frontal convexity	BIBD	[32]
6/F	67	5.7	Dysarthria	Pseudobulbar palsy, nonfluent aphasia	Posterior portion of frontal convexity	NIFID	[19,29,31]
7/M	29	8	Disinhibition	Behavioral abnormality	Posterior portion of frontal convexity, temporal pole	NIFID	[19,33]
8/M	39	13	Apathy, behavioral abnormality	Behavioral abnormality	Temporal pole, temporal base	aFTLD-U	
9/F	30	15	Behavioral abnormality, memory impairment	Behavioral abnormality	Frontal convexity	Unclassifiable	[25,30]

Additional features: cases 1, 2, 4 and 6 were associated with parkinsonism. Case 6 was associated with lower motor neuron signs in the terminal stage.
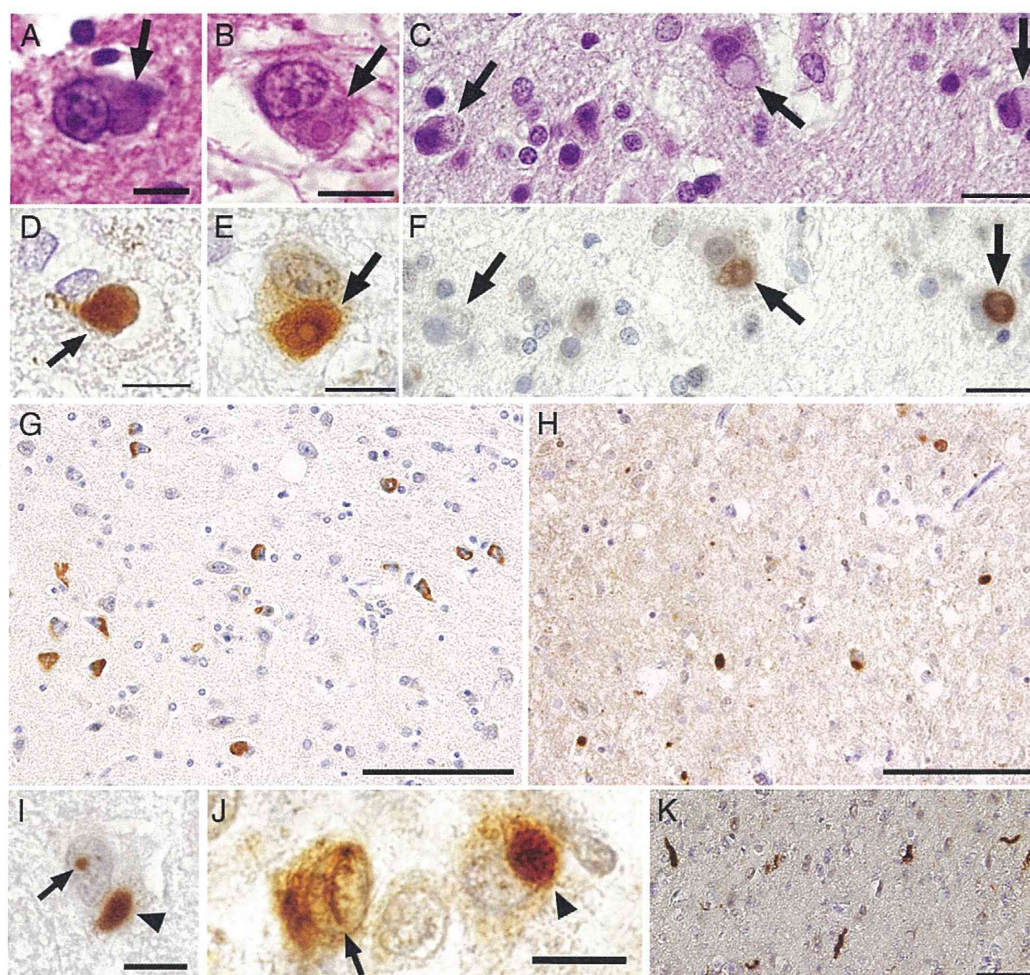
Fig. 1. A: hematoxylin and eosin (HE) staining of the frontal cortex of a BIBD case, case 5. The neuronal cytoplasmic inclusion (NCI) has a thin basophilic rim (arrow). B: HE staining of the hippocampus of an NIFID case, case 6. The NCI (arrow) in a pyramidal neuron contains a distinct eosinophilic core. C: HE staining of the frontal cortex of another NIFID case, case 7. The arrows indicate NCIs. The central compartments of NCIs were faintly stained and outlined by relatively intense staining. D: an α-internexin positive NCI in case 6. Panels E through K are immunostaining for FUS [86–213]. E: a FUS positive inclusion with a core (arrow) in the hippocampus of an NIFID case, case 6. The core is stained only weakly. F: the section shown in C was destained and restained immunohistochemically for FUS [86–213]. Immunoreactivity of the inclusions for FUS is variable (arrows). G: multiform – annular, crescent, tangle-like and oval – NCIs in the frontal cortex of a BIBD case, case 5. H: round NCIs in the frontal cortex of an a-FTLD-U case, case 8. I: a round neuronal intranuclear inclusion (NII) (arrow) and an NCI (arrowhead) in the hippocampus of an NIFID case, case 7. J: the hippocampus of an aFTLD-U case, case 8. The arrow and arrowhead indicate a vermiform NII and a NCI (arrowhead), respectively. K: FUS positive dystrophic neurites (DNs) in case 9. Scale bars are 10 μm in A, B, D, E, I and J; 20 μm in C and F; 50 μm in G and H; 50 μm in K.

the nuclei (Figs. 2A, B and 3A insert). When we raised the concentration of the antibody, both NCIs and the nuclei became positive (Fig. 3A). In some patients, however, only NCIs were labeled even at the highest possible concentration of the antibody, at which high background staining began to cloud the specific labeling. In type N, positive staining of the nuclei appeared first, and then NCIs became positive at higher concentrations of the antibody. Similarly to type I, only the nuclei were labeled in some patients, even at the highest possible concentration of the antibody. Figs. 2C, D and 3B illustrate nuclear labeling in the absence of NCIs staining at a low concentration of an antibody.

The staining patterns of each antibody in all cases are summarized in Table 3. In all cases, two antibodies to the middle portions of FUS, anti-FUS [52–400] and anti-FUS [86–213], showed the type I pattern. These two antibodies, particularly anti-FUS [86–213], were those most frequently used in the literature. While anti-FUS [250–300] did not work well in a number of cases, this antibody also showed the type I pattern when it worked. Antibodies to the carboxyl terminal portions, anti-FUS [400–450] and anti-FUS [500–526], on the other hand, showed

the type N pattern in most, if not all, cases. The staining pattern of the two antibodies to the amino-terminal portion, anti-FUS [1–50] from different sources, varied among cases but was type I in both NIFID cases and type N in 4 of 5 BIBD cases.

4. Discussion

Whereas an accurate incidence of the disease cannot be determined by examination of an institutional brain collection, FTLD-FUS comprised approximately 14% of all FTLD in the present study. If one considers the selection bias for autopsies of clinically unusual FTLD cases, such frequencies may be fairly comparable with or only a little more than those reported in western countries [6,7,9,12]. A clear difference, however, does exist in the ratios of FTLD-FUS subtypes. In this FTLD-FUS archive, only one case had pathology consistent with aFTLD-U, which is the most common subtype of FTLD-FUS in western countries [5,13,14]. Again, a selection bias for autopsies of cases with movement disorders might be present and increase BIBD and NIFID relative to aFTLD-U. However, the difference may be too large to be
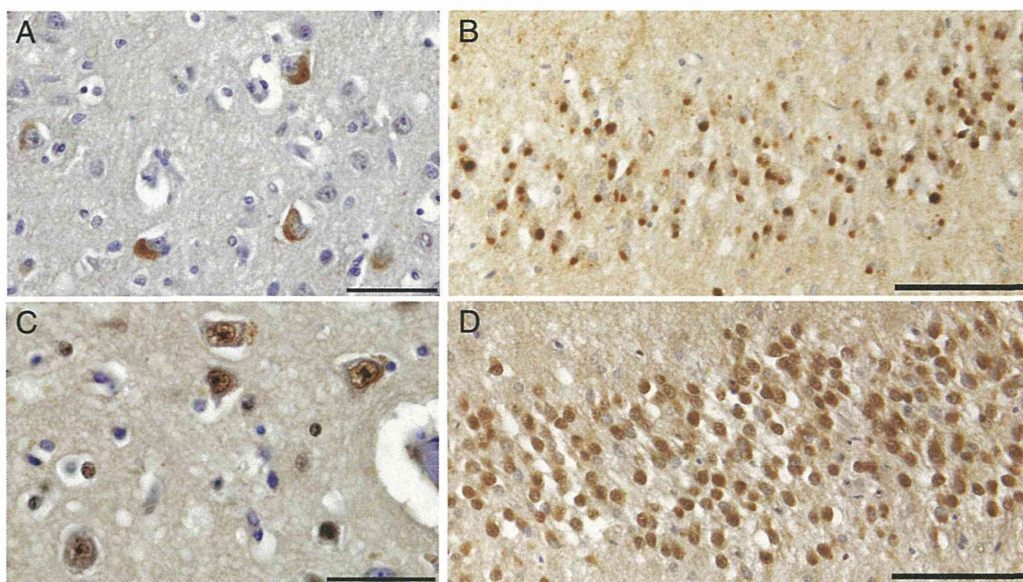
Fig. 2. A, C: High power photomicrographs of the frontal cortex, layer III, of a BIBD case. B, D: The granular cell layer of the hippocampal dentate gyrus of an NIFID case. The anti-FUS [52–400] antibody labels only NCIs but not the nuclei at a concentration of 13 μg/ml (A, B), showing the type I pattern. The anti-FUS [400–450] antibody hardly stains NCIs but well stains the nuclei at a concentration of 50 μg/ml (C, D), showing the type N pattern. Scale bars are 50 μm (A, C) and 100 μm (B, D).

explained by such a bias because nearly half of the cases in this archive lacked clinically evident movement disorders (Table 2). Furthermore, this aFTLD-U case did not seem to be typical in that NIIs occurred only occasionally, even in the dentate gyrus of the hippocampus. Such a feature rather suggests some pathological overlap with BIBD in this case. An ethnic difference present in sporadic diseases, i.e., a distinct subtype frequency, may suggest that some yet unidentified genetic background or environmental factors influence the pathophysiology of FTLD-FUS.

Comparison of FUS positive inclusions between BIBD and NIFID largely confirmed observations by Mackenzie et al. [5]. In one of the cases of NIFID, case 6, the NCIs bearing eosinophilic cores were α-internexin negative [19] but FUS positive. The cores themselves were negative or weakly positive for FUS, however. Even after extensive observation, we were not able to find NIIs in case 6. NIIs were present in case 7 but this case lacked NCIs with eosinophilic cores. While both cases with and without NCIs with eosinophilic cores have been classified into NIFID [11], it seems that there still remains a possibility that NIFID consists of heterogenous diseases [18]. At any event, the limited number of cases did not permit further analyses in the present study.

At present, the mechanisms by which α-internexin and neurofilament proteins are deposited in some NCIs in FTLD-FUS remain unclear. Neuronal intermediate filament proteins, such as α-internexin, are known to be accumulated in the neuronal cytoplasm in many degenerative diseases [16], as well as in a model of axonal transport disruption by brain injury [34,35]. In this study, we found α-internexin positive NCIs in two BIBD, cases 1 and 4, a finding consistent with a previous report [5]. In addition, we saw diffuse cytoplasmic accumulation of α-internexin in the brain regions with degenerative changes. It has to be noted that such intense cytoplasmic staining was often indistinguishable from labeling of NCIs. It seems that NIFID and other subtypes of FTLD-FUS can not always be distinguished with certainty by α-internexin immunohistochemistry.

In the present study, we have revealed that the nuclei and NCIs/DNs have different immunohistochemical profiles, depending on the portions of FUS that are recognized by the antibodies. Antibodies to the carboxyl terminal portion of FUS show a nuclei-dominant staining pattern, a finding which indicates that the carboxyl terminal portion of the FUS molecule is easier to be accessed in the nuclei than in NCIs/DNs. Antibodies to the middle portion of FUS, on the other hand, show a NCIs/DNs-dominant staining pattern, indicating that the middle portion can be more readily accessed in NCIs/DNs than in the nuclei. DNs in the atypical case, case 9, with DNs-dominant FUS accumulation in the cerebral cortex also showed a similar immunohistochemical profile to NCIs/DNs in other cases. A weakness of these findings is that they rely on the results obtained in formalin-fixed, paraffin-embedded tissue sections treated with heat for antigen retrieval. Sensitivity of FUS immunohistochemistry is known to be decreased by prolonged formalin fixation. All brains employed in the present study were formalin-fixed for long periods that varied from several weeks to months.

However, in well-controlled and lightly-fixed tissues that do not require antigen retrieval pretreatment, the immunohistochemical profiles of FUS also differ in normal brain between the nucleus and the dendrites/synapses [36]. The immunohistochemical profile of FUS in NCIs/DNs is close to that of cytoplasmic FUS in the dendrites/synapses. It may be noteworthy that cytoplasmic FUS is increased in pathological conditions [36], and that translocation of FUS from the nucleus to cytoplasm has been implicated in the pathogenesis of FUS proteinopathy [7,21,37]. The results of this study seem to be consistent with these recent observations. At present, whether such a difference is derived from a conformational change, or complex formation with other molecules, remains unknown. Though the low incidence of FUS proteinopathy causes difficulty in analyzing the biochemical natures of FUS abnormalities, further efforts have to be made to unveil the molecular basis for FUS accumulation in diseased conditions.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jns.2013.08.035.
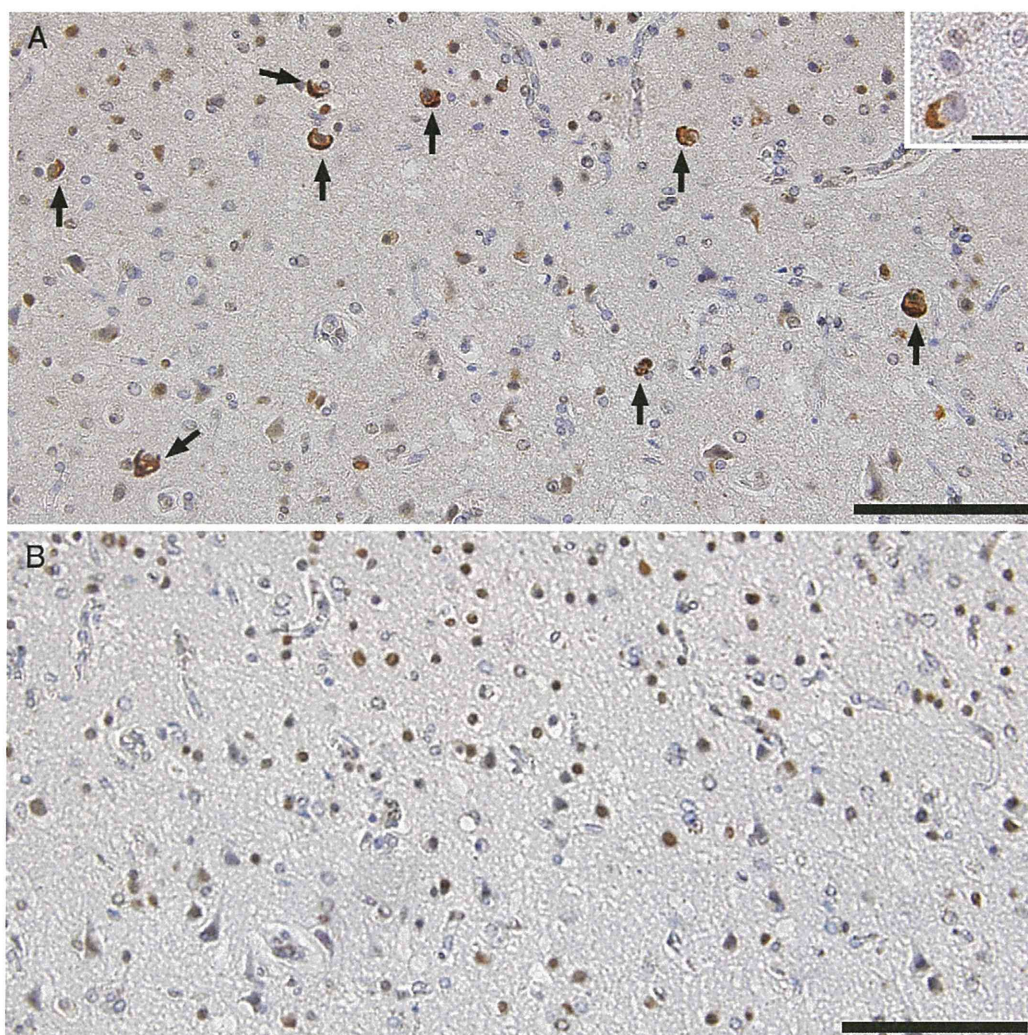
Acknowledgment

Fig. 3. Layers II and III of the frontal cortex of an NIFID case. A: Immunostaining with anti-FUS [86–213] at a concentration of 6.25 μg/ml. Both NCIs (arrows) and the nuclei are positive, but NCIs are more intensely stained than the nuclei. The insert shows a photomicrograph taken from a nearby section stained at a lower concentration (1.25 μg/ml) of the same antibody. Only the inclusion is positive and the nuclei are not labeled. Thus, this antibody shows the type I pattern in this case. B: Immunostaining with anti-FUS [500–526] at a concentration of 10 μg/ml. Only the nuclei are stained positively and NCIs remain unstained at this concentration of the antibody, showing the type N pattern. Scale bars are 100 μm (A, B) and 20 μm (*insert* in A).

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ORIGINAL ARTICLE

TDP-43 associates with stalled ribosomes and contributes to cell survival during cellular stress

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Abstract

TAR DNA-binding protein 43 (TDP-43) has emerged as an important contributor to amyotrophic lateral sclerosis and frontotemporal lobar degeneration. To understand the physiological roles of TDP-43 in the complex translational regulation mechanisms, we exposed cultured cells to oxidative stress induced by sodium arsenite (ARS) for different periods of time, leading to non-lethal or sublethal injury. Polysome profile analysis revealed that ARS-induced stress caused the association of TDP-43 with stalled ribosomes via binding to mRNA, which was not found under the steady-state condition. When the cells were exposed to short-term/non-lethal stress, TDP-43 associating with ribosomes localized to stress granules (SGs); this association was transient because it was immediately dissolved by the removal of the stress. In contrast, when the cells were exposed to long-term/sublethal stress, TDP-43 was excluded from SGs and shifted to the heavy fractions independent of any binding to mRNA. In these severely stressed cells, biochemical alterations of TDP-43, such as increased insolubility and disulfide bond formation, were irreversible. TDP-43 was finally phosphorylated via the ARS-induced c-jun N-terminal kinase pathway. In TDP-43-silenced cells, stalled mRNA and poly (A)+ RNA stability was disturbed and cytotoxicity increased under sublethal stress. Thus, TDP-43 associates with stalled ribosomes and contributes to cell survival during cellular stress.

Keywords: amyotrophic lateral sclerosis, apoptosis, frontotemporal lobar degeneration, oxidative stress, stress granule, TDP-43.

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the relentless degeneration of both upper and lower motor neurons and the presence of intraneuronal aggregates in the affected tissue. Although the exact mechanisms underlying ALS remain unclear, both genetic predisposition and environmental risk factors are thought to contribute to the development of the disease. In addition to increasing age, several potential environmental stresses, including oxidative stress, mitochondrial dysfunction, excitotoxicity, and endoplasmic reticulum stress, are speculated to be involved in the disease (Cleveland and Rothstein 2001; Bruijn *et al.* 2004). However, it has not been elucidated which of the processes involved in the stress response is the most important for triggering cell death in the disease.

Recently, two genes encoding DNA/RNA-binding proteins, TAR DNA-binding protein 43 (TDP-43) and fused in sarcoma/translated in liposarcoma (FUS/TLS, hereafter referred to as FUS), were reported to be ambiguous causes of FALS (Kabashi *et al.* 2008; Sreedharan *et al.* 2008;

Kwiatkowski *et al.* 2009; Vance *et al.* 2009). These proteins have structural and functional similarities, including an RNA-recognition motif and a glycine-rich region, and belong to the group of heterogeneous nuclear ribonucleoproteins (hnRNPs). Moreover, TDP-43 and FUS are major components of proteinaceous inclusions observed in affected regions from patients with sporadic ALS or frontotemporal lobar degeneration (FTLD) (Arai *et al.* 2006; Neumann *et al.* 2006). Thus, the RNA metabolism that these proteins are