protein deposition remains unknown. This study strongly supports a seed-dependent mechanism for the formation of the intracellular protein aggregates. In the context of our propagation hypothesis, it will be crucial to inhibit not only the production of intracellular amyloid seeds but also their spread into the extracellular space. Vaccination against the intracellular amyloid proteins, such as α-syn (41) or Tau may be an effective approach, together with inhibition of intracellular amyloid filament formation by small molecular inhibitors, for the therapy of these diseases.

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Supplemental data

Seeded aggregation and toxicity of α-synuclein and tau: cellular models of neurodegenerative diseases

Takashi Nonaka, Sayuri T. Watanabe, Takeshi Iwatsubo, Masato Hasegawa

Supplemental figure legends

Figure S1. Time-course observation of cells transduced with Seed αS.
(A-B) Electron microscopic analyses of Seed αS used in this study with (B) or without (A) sonication before use. Scale bars, 200 nm.
(C) Confocal microscopic images of SH-SY5Y cells 1 day and 3 days after treatment with Seed-HA in the presence or absence of LA. Cells were stained with anti-HA (red), anti-PSer129 (green) and TO-PRO-3 (blue). Scale bars, 50 μm.
(D) Confocal microscopic images of SH-SY5Y cells transfected with or without pcDNA3-αsyn (WT) 1 day and 3 days after treatment with or without Seed-HA. Cells were stained with anti-HA (red), anti-PSer129 (green) and TO-PRO-3 (blue). Scale bars, 50 μm.

Figure S2. Intracellular α-syn aggregate formation is dependent on the amount of α-syn seeds.
Cells were transfected with pcDNA3-αsyn (αsyn) and then transduced with different amounts of Seed-HA. After incubation for 3 days, cells were harvested and immunoblot analyses of the lysates was performed. α-Syn differentially extracted from the cells with Tris-HCl (TS), Triton X-100 (TX), and Sarkosyl (Sar), and the pellet (ppt), were probed using anti-HA (A) and anti-PSer129 (B). Immunoreactivity of phosphorylated α-syn in the TX-insoluble fraction was quantified using anti-PSer129. The results are shown in (C).

Figure S3. α-Syn fibrils seed intracellular aggregate formation of plasmid-derived α-syn, but not tau.
Cells were transfected with empty plasmid (none), pcDNA3-αsyn (αsyn) or pcDNA3-tau 3R1N (3R1N) and then treated with Seed-HA. After incubation for 3 days, cells were harvested and immunoblot analyses were performed. Proteins differentially extracted from the cells with Tris-HCl (TS), Triton X-100 (TX), and Sarkosyl (Sar), and the pellet (ppt), were probed using

anti-HA (A), anti-PSer129 (B), anti-T46 (C) and anti-PS396 (D).

Figure S4. Apoptosis is not induced in cells harboring intracellular α-syn aggregates.
(A) TUNEL staining of cells treated without (none) or with 1 μM staurosporine (stsp) for 8 hr and cells transfected with or without pcDNA3-αsyn (WT) 3 days after treatment with or without Seed αS. Cells were stained with TUNEL reagent (green), anti-PSer129 (red) and TO-PRO-3 (blue). Scale bars, 100 μm.
(B) The measurement of caspase-3 activity in cultured cells. Cells were transfected with empty plasmid (none) or pcDNA3-αsyn (WT) and then treated with or without Seed αS. After incubation for 3 days, cells were harvested and caspase-3 activity in the lysates was measured using Ac-DEVD-MCA as a substrate. Cell lysate treated with stsp was used as a positive control.

Fig. S5. Introduction of tau 4R1N monomer and fibril seed by Lipofectamine.

Purified recombinant tau (4R1N monomer; 2 μg) and filaments (Seed 4R1N, 2 μg) were sonicated and then incubated with Lipofectamine (LA). The protein-LA complexes were dispersed in opti-MEM and added to SH-SY5Y cells expressing pcDNA3-tau 4R1N. After 48 hr of culture, the cells were collected in the presence of 0.25% trypsin, and tau proteins were differentially extracted from the cells with Tris-HCl (TS), Triton X-100 (TX) and Sarkosyl (Sar), and the pellet (ppt), were probed with anti-T46 (upper), anti-HT7 (middle) and anti-PS396 (lower).

Fig. S1 Nonaka et al

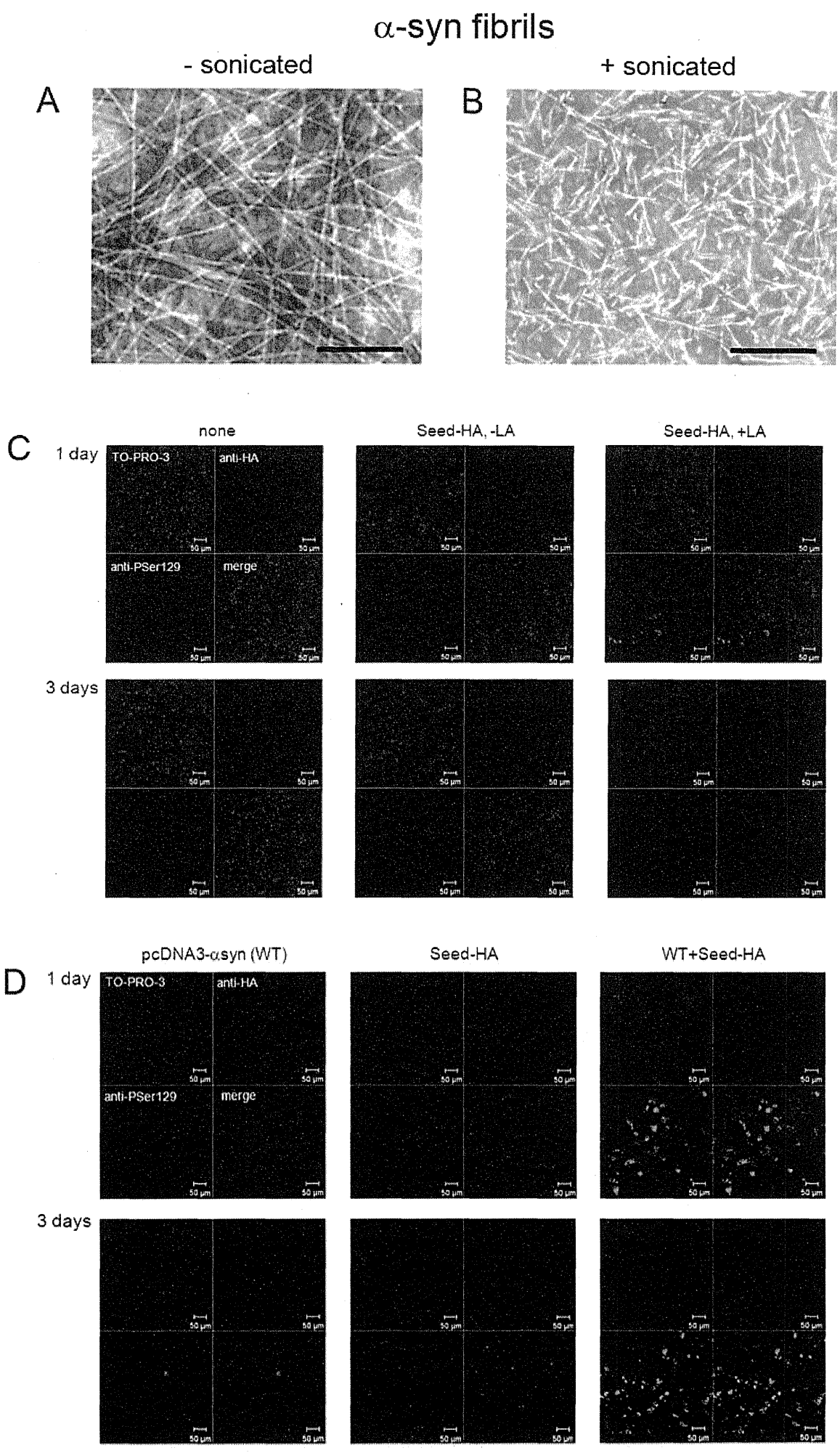
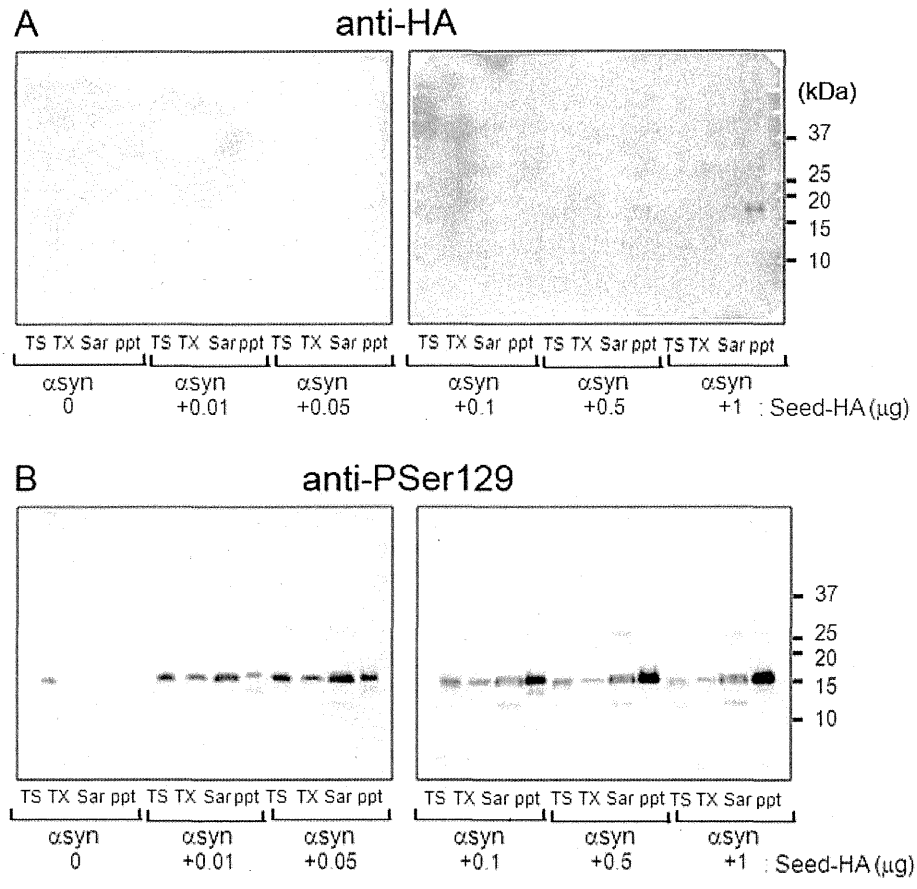


Fig. S2 Nonaka et al



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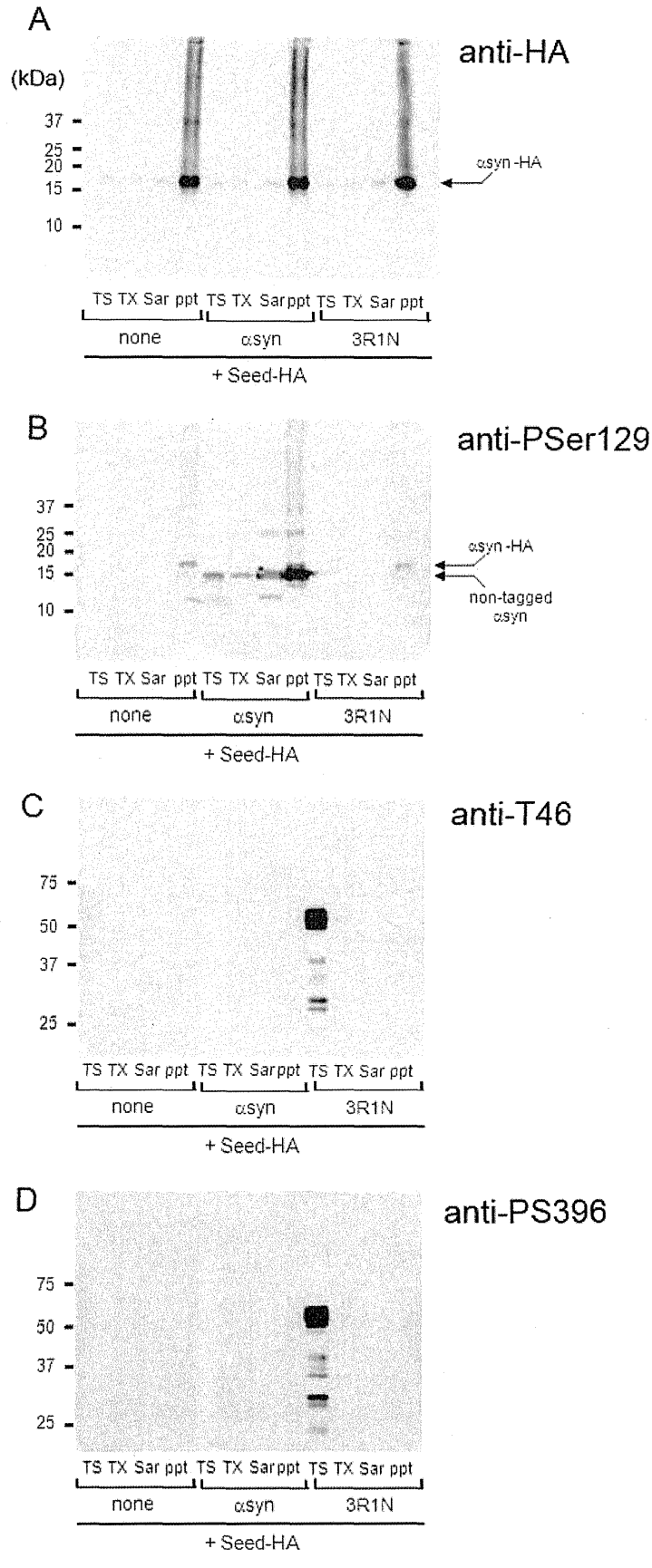

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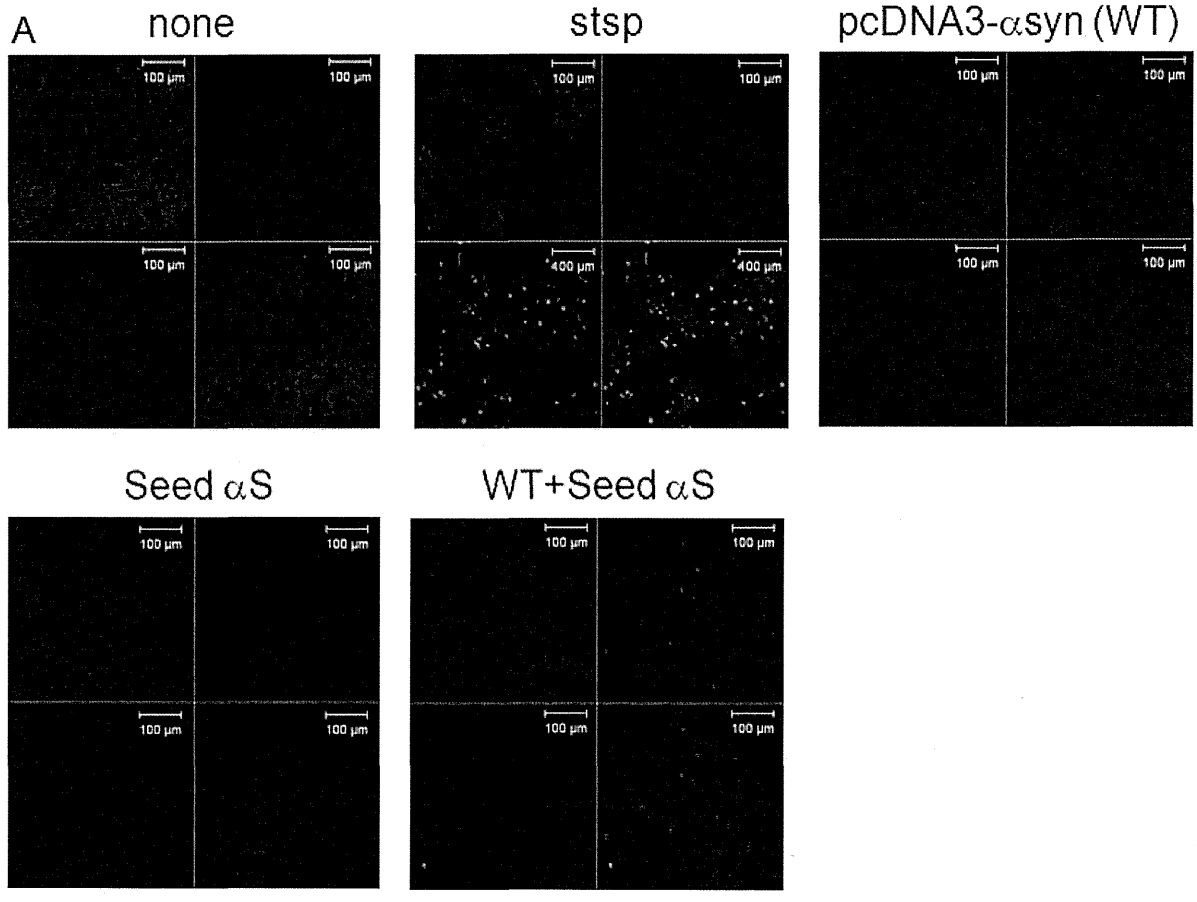


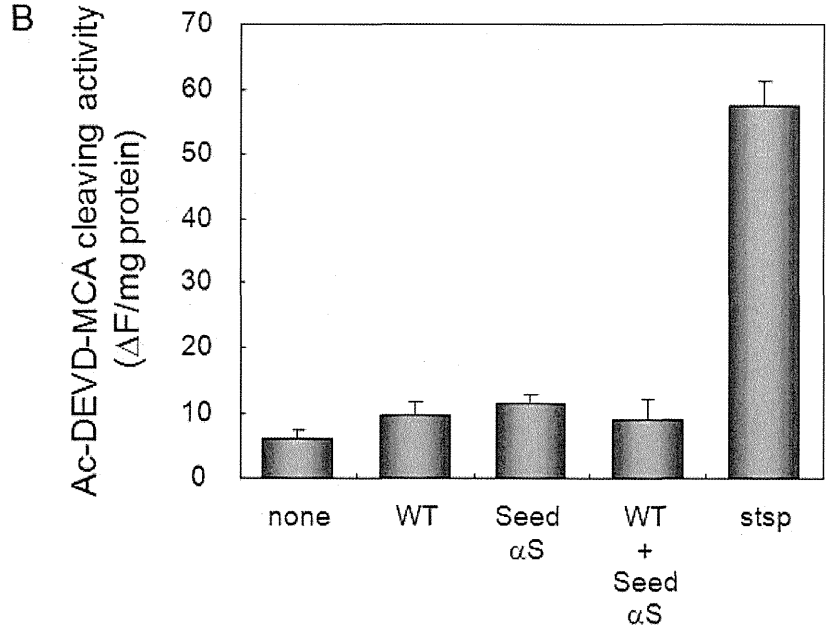
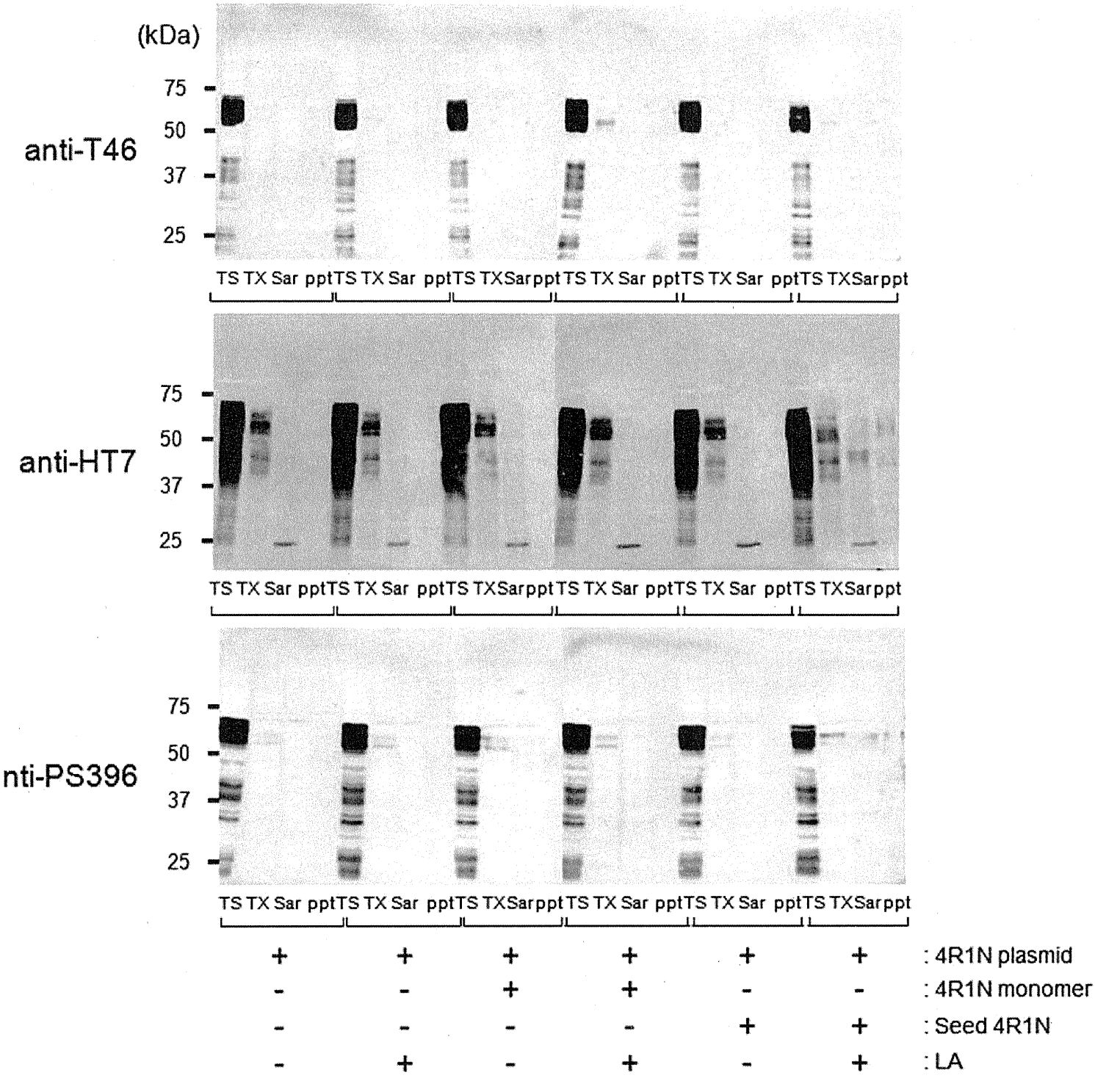


Fig. S5 Nonaka et al



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Clinicopathological characteristics of FTLD-TDP showing corticospinal tract degeneration but lacking lower motor neuron loss

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ABSTRACT

The presence of frontotemporal lobar degeneration with TDP-43-positive inclusions (FTLD-TDP) showing corticospinal tract (CST) degeneration but lacking lower motor neuron (LMN) loss has been reported, and the term primary lateral sclerosis (PLS) is used to distinguish motor neuron disease (MND) of these cases from amyotrophic lateral sclerosis (ALS). To date, however, details of clinicopathological findings of FTLD-MND-PLS type (FTLD-MND-P) have not been reported. We evaluated medical records and histopathological findings of ten cases of FTLD-MND-P, in comparison with those of six FTLD-MND-ALS type (FTLD-MND-A) cases. The mean age at onset and disease duration of FTLD-MND-P cases were 54 and 12 years, respectively. The first symptoms were frontotemporal dementia showing behavioral abnormality and/or personality change in five cases, semantic dementia in three cases, progressive non-fluent aphasia in one case, and auditory hallucination in one case. Upper motor neuron signs were clinically identified in six of the ten cases. There were no LMN signs throughout the clinical course in any case. Histopathologically, there was no obvious LMN loss or Bunina bodies in the hypoglossal nucleus or spinal cord in any case, whereas the CST was involved in all cases. The cerebral cortex of the six cases showed type 1 of TDP-43 histology defined by Cairns et al., whereas three cases showed type 3 histology, and one case showed type 2 histology. In all cases, TDP-43 positive neuronal cytoplasmic inclusions were absent or rare in the LMNs, while TDP-43 positive round structures were frequently identified in the neuropil of the spinal cord anterior horn in some cases. This study clarified that FTLD-MND-P cases have characteristic clinicopathological features distinct from those of FTLD-MND-A.

1. Introduction

Clinical phenotypes of FTLD-TDP include frontotemporal dementia (FTD) showing behavioral abnormality and/or personality change, semantic dementia (SD), and progressive non-fluent aphasia (PA). The most common subtype of FTLD-TDP is FTD [1,2], and a proportion of patients with FTD develops motor neuron disease (MND), which usually refers to amyotrophic lateral sclerosis (ALS) [3].

Definitions of FTLD with MND (FTLD-MND) and ALS with dementia (ALSD) are dependent on which symptoms present first [4]. The disease duration of FTLD-MND is about 44 months, whereas that of ALSD is about 34 months [5]. Exceptionally, an FTLD-MND case showing a disease duration of 11 years and TDP-43-positive neuronal cytoplasmic inclusions (NCIs) in the cerebral cortex was reported [6,7]. In FTLD-MND, personality changes are usually mild [8], and SD or PA is hardly seen. The bulbar regions and upper limbs are preferentially affected, and the hypoglossal nucleus usually shows neuronal loss [8].

There are four subtypes of TDP-43 histology in the cerebral cortex of FTLD-TDP [9]. Type 1 histology is characterized by an abundance of dystrophic neurites (DNs) predominantly in the superficial cortical

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layers, with few NCIs. Glial cytoplasmic inclusions (GCIs) are rare. Type 2 histology shows abundant NCIs in both the superficial and deep cortical layers with few DNs. GCIs are frequently seen in the gray and white matter. Type 3 histology presents with mixed or intermediate histology of types 1 and 2, namely abundant DNs and NCIs predominantly in the superficial cortical layers. Neuronal intranuclear inclusions (NIIs) are occasionally seen, and GCIs are often present. These histological subtypes correspond to the distinct patterns of immunoblot bands of cleaved TDP-43 [10]. Josephs et al. reported that mutations of the proguranulin gene were seen in 35% of cases showing type 3 histology [1] (type 1 histology defined by Mackenzie et al. [11]). FTLD-MND cases show type 2 or 3 histology [9,12]. In general, type 3 is the most common of the four types [1,9,12,13]. The disease duration increases from type 2 to 3, and to 1 [1,12,14], and the duration of type 1 is reported to range from six [14] to ten [1] years.

The corticospinal tract (CST) is the largest and most important descending fiber system, and the CST fibers arise not only from the PMC, but also from the premotor cortex, supplementary motor area, and parietal areas [15]. Recently, the presence of FTLD-TDP showing CST degeneration but lacking LMN loss was reported [1,16,17], and the term primary lateral sclerosis (PLS) is used to distinguish MND of these cases from ALS [1,16]. Josephs et al. reported that the disease duration in two cases of FTLD-MND-PLS type (FTLD-MND-P) was six years and seven years, respectively [16]. They subsequently showed that there were only two cases of FTLD-MND-P among 39 FTLD-TDP cases, and the cerebral cortex of these cases presented with type 1 histology [1] (type 2 histology defined by Mackenzie et al. [11]). In contrast, Yokota et al. recently reported that FTLD-MND-P is not rare among FTLD-TDP cases in Japan [17]. Because details of the clinicopathological findings of FTLD-MND-P have not been reported to date, we evaluated the clinical and histopathological findings focusing on the motor system in ten FTLD-MND-P cases, and compared them with those of six FTLD-MND-ALS type (FTLD-MND-A) cases.

2. Materials and methods

2.1. Subjects

There were 29 cases of FTLD-TDP including 13 women and 16 men pathologically examined in Tokyo Institute of Psychiatry from 1974 to 2009. Some of these cases were reported previously [17–22]. All patients fulfilled the international clinical and pathological diagnostic criteria [3,23]. The clinical information and TDP-43 histology of these 29 cases are shown in Table 1. There were no cases showing a family history of FTLD or type 4 TDP-43 histology. The numbers of cases showing types 1, 2 and 3 were 10, 14 and 5, respectively. Although the cases showing type 3 were rare in our series, this tendency may be partly explained by the low frequency of familial cases of FTLD in Japan [24,25]. The mean disease duration of each type was 12.7 years, 2.7 years and 6.6 years, respectively. There were four cases of ALSD that initially showed LMN signs such as muscle atrophy and weakness, followed by dementia. Conversely, there were six cases of FTD with ALS that initially presented with dementia, followed by LMN signs. These cases of ALSD and FTD with ALS showed LMN loss, formation of Bunina bodies, and type 2 TDP-43 histology while one case of FTD with ALS showed type 3 histology. Among 14 cases showing type 2 histology in our institution, all but one case showed variable degrees of LMN loss.

Table 1
FTLD-TDP cases in our institution.

Type of TDP-43 histology	Mean disease duration, y	Clinical phenotype	Number of cases
Type 1	12.7	SD	7 (3)
		FTD	3 (3)
Type 2	2.7	FTD with ALS	5 (0)
		ALSD	4 (0)
		FTD	2 (0)
		SD	2 (0)
		PA	1 (1)
Type 3	6.6	FTD	3 (2)
		FTD with ALS	1 (0)
		unclassifiable	1 (1)

SD semantic dementia, *FTD* frontotemporal dementia, *ALS* amyotrophic lateral sclerosis. *ALSD* ALS with dementia, *PA* progressive non-fluent aphasia. Parenthesis shows the number of FTLD-MND-P cases.

In this study, we defined "FTLD-MND-P" as FTLD-TDP showing CST degeneration but lacking obvious LMN loss or Bunina bodies in the hypoglossal nucleus or spinal cord. Among the 29 FTLD-TDP cases examined in our institution, we first excluded four cases showing type 1 histology because the available tissue was limited. Subsequently, we identified ten FTLD-MND-P cases (cases 1–10, shown in Table 2). At our institution, there was only one FTLD-TDP case showing preservation of both the CST and LMNs in the brain and spinal cord. This case showed an absence of UMN signs throughout the total clinical course of seven years and presented with type 3 TDP-43 histology in the cerebral cortex. For comparison, we examined six FTLD-MND-A cases (cases 11–16, shown in Table 2).

2.2. Conventional neuropathology

Brain tissue samples from all subjects were fixed postmortem with 10% formalin and embedded in paraffin. Hemispheric sections (10 μm thick) were prepared from the frontal, temporal, parietal, and occipital lobes. Sections of midbrain, pons, medulla oblongata, cerebellum, and cervical, thoracic, and lumbar cord were also prepared when available. In case 3 only, hemispheric sections of the cerebrum were not available. These sections were stained by the hematoxylin–eosin (HE), Klüver–Barrera (KB), Holzer, methenamine silver, Bodian, and Gallyas–Braak methods. A genetic study could not be done because only formalin-fixed and paraffin-embedded tissues were available.

2.3. Assessment of the cerebral cortex and CST

The severity of neuronal loss and gliosis in the cerebral cortex was assessed on HE-, KB-, Bodian- and Holzer-stained sections. The PMC was identified as the cortex where Betz cells were identified. The CST degeneration in the subcortical white matter of the PMC, posterior limb of the internal capsule, cerebral peduncle, and spinal cord was assessed by the evidence of loss of myelin and axons, glial proliferation, and the presence of macrophages, and indicated as – (absent) or + (present). The CST degeneration at the level of the medulla oblongata was assessed according to the grading system of the previous study [17], and was indicated as –: no degeneration (no myelin pallor), 1+: mild degeneration (slight myelin pallor without atrophy of the pyramid), 2+: moderate degeneration (evident myelin pallor with slight atrophy of the pyramid), and 3+: severe degeneration (evident myelin pallor with severe atrophy of the pyramid).

2.4. Immunohistochemistry

Antibodies used in immunohistochemistry are shown in Table 3. Sections from the frontal, temporal and anterior parietal lobes, brainstem, cerebellum, and cervical, thoracic, and lumbar cord were examined using antibodies to TDP-43. Cystatin C immunoreactivity was examined in the brainstem and spinal cord. Deparaffinized sections were incubated with 1% H_2O_2 in methanol for 30 min to eliminate endogenous peroxidase activity in the tissue. When using anti-TDP-43C [405–414], sections were pretreated by autoclaving for

Table 2
Clinical features of the FTLD-MND-P cases (cases 1–10) and FTLD-MND-A cases (cases 11–16).

Case no./sex	Age of onset, y	Onset age of LMN signs, y	Onset age of UMN signs, y	Age at death, y	Disease duration, y	Clinical course	UMN signs	Clinical *diagnosis*
1/M	47	–	48	50	2.7	FTD, UMN signs	Hyperreflexia (rt), Hemiparesis (rt)	SD?
2/M	75	–	N	81	5.8	PA	N	SPA
3/F	49	–	N	57	8	FTD	N	AD
4/M	52	–	60	61	10	SD, FTD, UMN signs, Parkinsonism	Hyperreflexia (rt), Spasticity (rt), Babinski's sign (rt)	Pick
5/M	52	–	N	63	11	FTD	N	Pick
6/F	48	–	54	60	12	Auditory hallucination, SD, FTD, UMN signs	Hyperreflexia (rt), Babinski's sign (rt)	Pick
7/F	58	–	72	72	14	SD, FTD, UMN signs	Babinski's sign	Pick
8/M	58	–	66	74	16	FTD, SD, Parkinsonism, UMN signs	Hyperreflexia (lt), Babinski's sign, Paralysis of upper limb (lt)	Pick
9/M	55	–	72	74	19	SD, FTD, Parkinsonism, UMN signs	Hyperreflexia, Ankle clonus	SPA
10/F	49	–	N	70	21	FTD	N	Pick
11/M	60	61	–	61	1.7	Memory impairment, FTD, LMN signs	–	Dementia with MND
12/M	63	63	64	65	1.7	FTD, LMN and UMN signs	Hyperreflexia, Babinski's sign	ALSD
13/M	49	50	50	52	3	FTD, SD, LMN and UMN signs	Hyperreflexia	Dementia with MND
14/F	39	39	41	42	3.2	FTD, LMN and UMN signs	Hyperreflexia	Dementia with MND
15/F	54	57	57	58	4	Memory impairment, FTD, LMN and UMN signs	Hyperreflexia	Dementia with MND
16/F	64	70	70	70	6	FTD, LMN and UMN signs	Hyperreflexia	Dementia with MND

–: absent, N: not recorded, rt: right side (or right side predominant), lt: left side (or left side predominant), *LMN* lower motor neuron, *UMN* upper motor neuron, *FTD* frontotemporal dementia, *PA* progressive non-fluent aphasia, *SD* Semantic dementia, *AD* Alzheimer's disease, *Pick* Pick's disease, *SPA* Slowly progressive aphasia, *MND* motor neuron disease, *ALSD* amyotrophic lateral sclerosis with dementia. In the column of Clinical course, the symptoms are described in the order that they appeared.

10 min in 10 mM sodium citrate buffer at 120 °C. After washing sections with 0.01 M phosphate buffered saline (PBS, pH 7.4) three times for 10-min each, the specimens were blocked with 10% normal serum. Sections were incubated overnight at 4 °C with one of the primary antibodies in 0.05 M Tris–HCl buffer, pH 7.2. After washing three times for 10-min each in PBS, 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 the chromogen. Sections were counterstained with hematoxylin.

2.5. Confocal microscopy

Double labeling immunofluorescence for TDP-43C [405–414] and ubiquitin (mouse, monoclonal, clone MAB1510; 1:200, Millipore, Temecula, CA, USA), and for TDP-43C [405–414] and p62-N (guinea pig, polyclonal; 1:500, PROGEN Biotechnik GmbH, Heidelberg, Germany) was performed on the spinal cord specimen from case 10. After washing with Tx-PBS for 30 min, sections were incubated for 2 h at room temperature in a cocktail of fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG (1:100, Millipore, Temecula, CA) and tetramethylrhodamine isothiocyanate (TRITC)-conjugated goat anti-rabbit IgG (1:100, Millipore). After washing, sections were incubated in 0.1% Sudan Black B for 10 min at room temperature and washed with Tx-PBS for 30 min. Sections were coverslipped with Vextashield (Vector Laboratories) and observed with a confocal laser microscope (LSM5 PASCAL; Carl Zeiss MicroImaging GmbH, Jena, Germany).

2.6. Assessment of other pathological changes

Neurofibrillary changes and senile plaques were evaluated by the Braak stage on Gallyas–Braak and methenamine silver-stained sections, respectively. Argyrophilic grains and Lewy pathology were evaluated on Gallyas–Braak silver-stained sections and alpha-synuclein immunostained sections, respectively. TDP-43 pathology was classified into types 1–4 according to the reported pathological criteria [9].

3. Results

3.1. Clinical features of FTLD-MND-P cases (cases 1–10)

The clinical features of cases 1–10 are summarized in Table 2. The mean age of onset and disease duration were 54 years (range 48–75 years) and 12 years (range 2.7–21 years), respectively. Six of the ten cases were male. The first symptoms were FTD in the five cases, SD in three cases, PA in one case, and auditory hallucination in one case. The UMN signs were recorded in six cases, and five of the six cases developed UMN signs in the middle or late stage of the disease. The laterality of the UMN signs was recorded in four cases. The UMN signs included hyperreflexia, spasticity, Babinski's sign, paralysis, and ankle

Table 3
Antibodies used in the immunohistochemistry.

Antibody	Type	Sourse	Dilution
Phosporylation-independent anti-TDP-43			
TDP43C [405–414]	Rabbit polyclonal	Made by Hasegawa et al. [10]	1:1000
Phosporylation-dependent anti-TDP-43			
pS409/410	Rabbit serum	Made by Hasegawa et al. [10]	1:1000
pS403/404	Rabbit serum	Made by Hasegawa et al. [10]	1:1000
Anti-α-synuclein			
Pα#64	Mouse monoclonal	Wako Chemical, Osaka, Japan	1:3000
Anti-cyctatin C	Rabbit polyclonal	Dako, Glostrup, Denmark	1:3000

Table 4
Pathological features of the FTLD-MND-P cases (cases 1–10) and FTLD-MND-A cases (cases 11–16).

			UMN pathology						LMN pathology			
				CST degeneration					Neuron loss			
Case No.	Brain weight (g)	Atrophy	Neuron loss in PMC	White matter of PMC	Internal capsule	Cerebral peduncle (right/left)	Pyramid (right/left)	Spinal cord (right/left)	HN	Spinal cord	Bunina bodies	Type of TDP-43 histology
1	1350	T>F (lt)	+	–	–	–/–	3+/3+	N/N	–	N	–	3
2	1050	T=F (lt)	+	+	–	–/–	1+/1+	N/N	–	N	–	2
3	890	N	N	N	–	+/N	1+/1+	N/N	–	N	–	3
4	1060	T>F	–	–	–	–/–	1+/1+	N/N	–	N	–	1
5	1040	T>F	+	–	–	N/–	N/2+	N/N	–	N	–	1
6	690	T>F (lt)	+	–	–	+/+	3+/3+	+/+	–	–	–	3
7	915	T>F	+	+	–	+/N	N/2+	N/N	–	N	–	1
8	920	T=F	+	–	+	+/+	3+/3+	+/+ [a]	±	– [a]	–	1
9	905	T>F (lt)	±	–	–	–/+	2+/2+	+/+	–	–	–	1
10	640	T>F (lt)	+	+	N	+/N	3+/3+	+/+	–	–	–	1
11	1340	-	N	-	-	-/-	1+/1+	+/+	+	+	+	2
12	1240	T (rt)	-	-	-	-/-	-/-	+/+	+	+	+	2
13	1260	-	+	-	-	-/-	1+/1+	+/+	+	+	+	2
14	1200	T = F	+	-	-	-/-	1+/N	N/N	+	N	+	3
15	N	F > T	+	-	-	-/-	1+/1+	+/+	+	+	+	2
16	1120	T = F	-	N	-	+/N	1+/1+	+/+	+	+	+	2

N: not able to evaluate, –: absent, ±: minimal, +: present, 1+: mild, 2+: moderate, 3+: severe, *T>F* temporal lobe-predominant atrophy, *F>T* frontal lobe-predominant atrophy, *T=F* temporal and frontal lobes were equally atrophic. *T* Temporal lobe atrophy, *lt* left side predominant atrophy, rt right side predominant atrophy, *PMC* primary motor cortex, *CST* corticospinal tract, *HN* hypoglossal nucleus. [a] Only the upper cervical cord was available. The UMN pathology in the cerebrum was evaluated on the right side in cases 1, 7 and 9, and on the left side in the other cases.

clonus. There were no LMN signs in any case. In cases 2, 3, 5 and 10, there were no descriptions indicating the presence or absence of UMN signs in the medical records, and in cases 7 and 9, there was no description regarding the laterality of the UMN signs. Signs of parkinsonism such as limb rigidity and hand tremor were recorded in three cases.

3.2. Neuropathological findings of FTLD-MND-P cases (cases 1–10)

A summary of the findings in cases 1–10 is shown in Table 4. The mean brain weight was 946 g (range 640–1,350 g). Only in case 3, the cerebrum could not be evaluated macroscopically because neither brain photographs nor hemispheric sections were available. In the other cases, the frontotemporal lobes showed temporal lobe dominant atrophy in cases 1, 4–7, 9 and 10, while the frontal and temporal lobes were equally atrophic in cases 2 and 8. The laterality of frontotemporal atrophy was seen in five cases. Atrophy of the precentral gyrus was generally mild, but only case 9 showed severe atrophy on the left side. The atrophy of the pyramid of the medulla oblongata was demonstrated in cases 1 and 5–10.

Microscopically, frontotemporal cortices showed temporal lobe dominant degeneration in cases 1, 4–7, 9 and 10, whereas frontal and temporal cortices were equally degenerated in other cases. In the temporal cortex of all cases except for case 2, moderate or severe neuronal loss was observed not only in the superficial layer but also in the deep layers, and myelin loss and gliosis were demonstrated in the adjacent white matter (Figs. 1a, b, d, 3a, b). There was neither obvious LMN loss nor Bunina bodies in the hypoglossal nucleus or spinal cord in any case (Fig. 2c, e). There were no cases showing neurofibrillary changes corresponding to Braak stage III–VI. Lewy-related pathology was seen only in case 10, which showed the limbic type [26]. Argyrophilc grains were not found in any case. The microscopic findings of the PMC and CST are described below.

3.2.1. PMC

The PMC was examined unilaterally in all cases except for case 3 in which the PMC could not be identified. Although the macroscopic atrophy of the left precentral gyrus was demonstrated in case 9, there were no samples from the left precentral gyrus in this case. In general, the PMC degeneration was mild when compared to other frontotemporal cortices. In detail, neuronal loss was not apparent in cases 4 and 9. In cases 2 and 5–8, mild neuronal loss and astrocytosis were demonstrated only in the superficial layer. In case 7, laminar astrocytosis was seen in the deep layers on Holzer staining. Cases 1 and 10 showed moderate neuronal loss in the superficial and deep layers (Fig. 1c). The Betz cells were sparse and showed atrophy in cases 1, 5–8 and 10. In contrast, loss of Betz cells was minimal in cases 2 and 9, and not apparent in case 4.

3.2.2. Subcortical white matter of the PMC

The subcortical white matter of the PMC was examined unilaterally in all cases except for case 3. Degeneration was demonstrated only in cases 2, 7 and 10 (Fig. 1a, b).

3.2.3. Posterior limb of the internal capsule

The posterior limb of the internal capsule was examined unilaterally in all cases except for case 10. Degeneration was demonstrated only in case 8 (Fig. 3a, b).

3.2.4. Midbrain

The cerebral peduncle was examined unilaterally in cases 3, 5, 7 and 10, and bilaterally in other cases. Degeneration was seen in the middle third of the cerebral peduncle corresponding to the CST in cases 3 and 6–10 (Figs. 2a, 3c), although cases 3, 7 and 10 showed more marked degeneration in the medial third of the cerebral peduncle corresponding to the frontopontine tract (Fig. 2a).

3.2.5. Medulla oblongata

The pyramid was examined unilaterally in cases 5 and 7, and bilaterally in other cases. Degeneration was demonstrated in all cases, and was severe in cases 1, 6, 8 and 10 (Fig. 2b), moderate (2+) in cases 5, 7 and 9, mild (1+) in other cases (Fig. 3d).

3.2.6. Spinal cord

The cervical, thoracic, and lumbar cord was available in cases 6, 9 and 10, and only upper cervical cord could be examined in case 8. The CST was involved in all cases (Fig. 2d).

3.2.7. TDP-43 pathology

Two kinds of phosphorylation-dependent antibodies against TDP-43 showed almost the same distribution and severity of abnormal structures in the brain and spinal cord. Anti-TDP43C [405–414], a

phosporylation-independent C terminal antibody, showed abnormal structures with weak staining of normal nuclei. Six of the eight cases (cases 4, 5 and 7–10) showed type 1 TDP-43 histology in the cerebral cortex (Fig. 1e, f), three cases (cases 1, 3 and 6) type 3 histology, and one case (case 2) type 2 histology. In the cases showing type 1 histology, abundant DNs with rare NCIs were demonstrated in the cerebral cortex, and GCIs were not observed in the gray or white matter including the CST. In the cases showing type 3 histology, abundant DNs and NCIs were observed in the cerebral cortex, and GCIs were sparsely seen. In case 2 showing type 2 histology, abundant NCIs without DNs were seen in the cerebral cortex, and GCIs were observed in the gray and white matter.

In all subtypes, TDP-43-positive structures were consistently observed in the frontotemporal cortices including the PMC, anterior parietal cortex, and striatum. The NCI in the Betz cell was demonstrated only in case 1. In the hypoglossal nucleus, there was no TDP-43 pathology in cases 1, 3 and 5–10, whereas only one NCI was observed in one section in case 4, and round structures in the neuropil were demonstrated in case 2. In the spinal cord anterior horn, there were rare NCIs in case 10, and rare DNs in cases 9 and 10. In cases 9 and 10, interestingly, TDP-43-positive round structures were frequently identified in the neuropil of the anterior horn (Fig. 2f). There was no TDP-43 pathology in the spinal cord in cases 6 and 8.

3.2.8. Double labeling immunofluorescence

Double labeling immunofluorescence was performed to clarify the characteristics of the round structures in the neuropil of the spinal cord anterior horn in case 10. Confocal immunofluorescence of ubiquitin and TDP-43C [405–414] showed colocalization in an NCI, and a round structure in the neuropil (Fig. 4). Similarly, immunofluorescence of p62 and TDP-43C [405–414] demonstrated colocalization in a DN, and a round structure in the neuropil, while an NCI immunoreactive only for p62 or for TDP-43 was also seen (Fig. 4).

3.3. Clinicopathological findings of FTLD-MND-A cases (cases 11–16)

The clinicopathological findings of cases 11–16 are summarized in Table 2. The mean age of onset and disease duration were 55 years (range 39–64 years) and 3.3 years (range 1.7–6 years), respectively. Three of the six cases were male. The first symptoms were FTD in four cases, and memory impairment in two cases. Case 12 developed FTD and LMN signs simultaneously, and was given a clinical diagnosis of ALSD. The longest duration of the appearance between dementia and LMN signs was six years (case 16). The UMN signs were recorded in five of the six cases, whereas the absence of the UMN signs was described in case 11. Parkinsonism was not recorded in any cases.

The mean brain weight was 1232 g (range 1120–1340 g). There was no apparent cerebral atrophy in cases 11 and 13. The frontotemporal lobes showed frontal dominant atrophy in case 15, while the frontal and temporal lobes were equally atrophic in cases 14 and 16. Case 12 showed atrophy only in the anterior temporal lobe

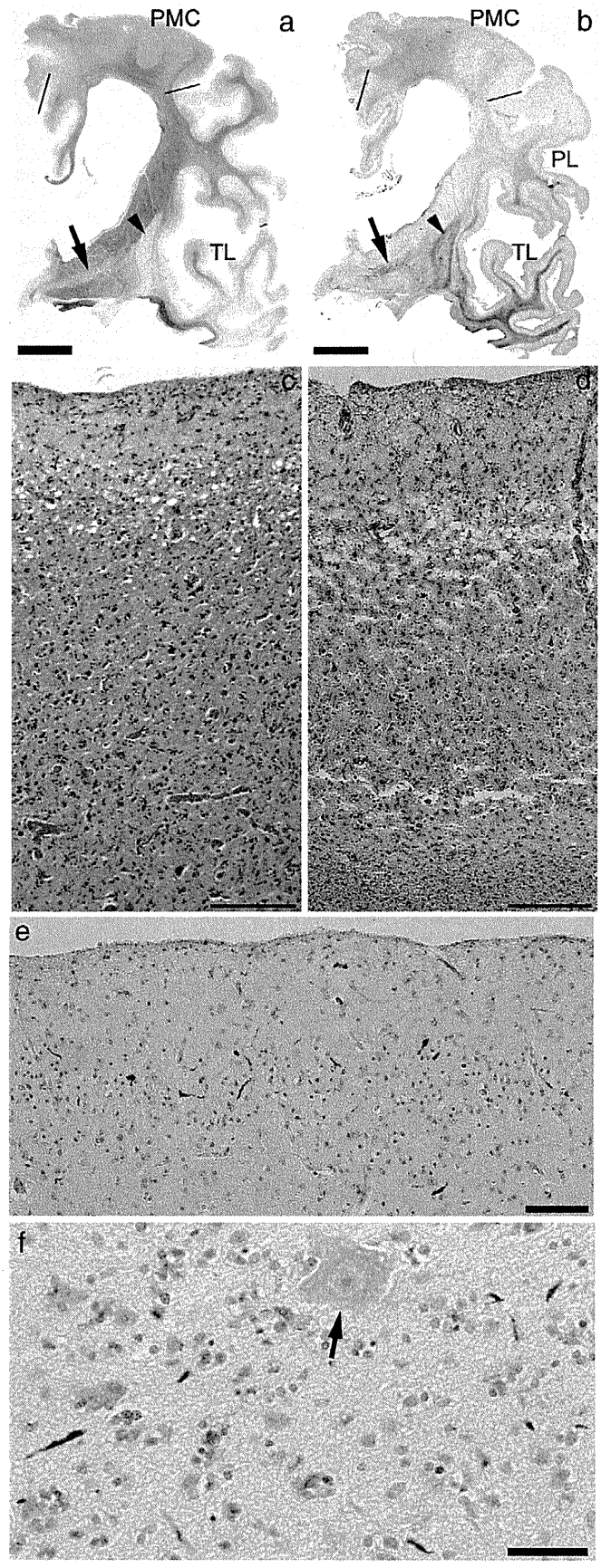

Fig. 1. (case 10) a and b are serial sections. a The left hemispheric section at the plane of the globus pallidus is shown. The area of cerebral cortex between the lines is the primary motor cortex (PMC) where Betz cells were identified. Cerebral atrophy was accentuated in the temporal lobe (TL). Enlargement of the lateral ventricle was evident, and the putamen (arrowhead) was atrophic. Mild myelin pallor was observed in the subcortical white matter of the PMC, whereas evident myelin pallor was seen in the subcortical white matter of the TL. The arrow indicates myelin pallor at the internal capsule (the anterior limb or genu). b Mild gliosis was demonstrated in the subcortical white matter of the PMC and anterior parietal lobe (PL), whereas evident gliosis was seen in the subcortical white matter of the TL. Gliosis was also seen in the internal capsule (arrow) and putamen (arrowhead). c Moderate neuronal loss was demonstrated in the superficial and deep layers of the PMC. d Severe neuronal loss was observed in the superficial and deep layers of the temporal cortex. e TDP-43 positive dystrophic neurites (DNs) were observed in the superficial layer of the PMC. f DNs were also demonstrated in the deep layer of the PMC. The arrow indicates a Betz cell. a Klüver–Barrera stain, b Holzer stain, c, d Hematoxylin–eosin stain, e, f anti-TDP43. *Scale bars* a, b 1 cm, c, d 200 μm, e 100 μm, f 50 μm.

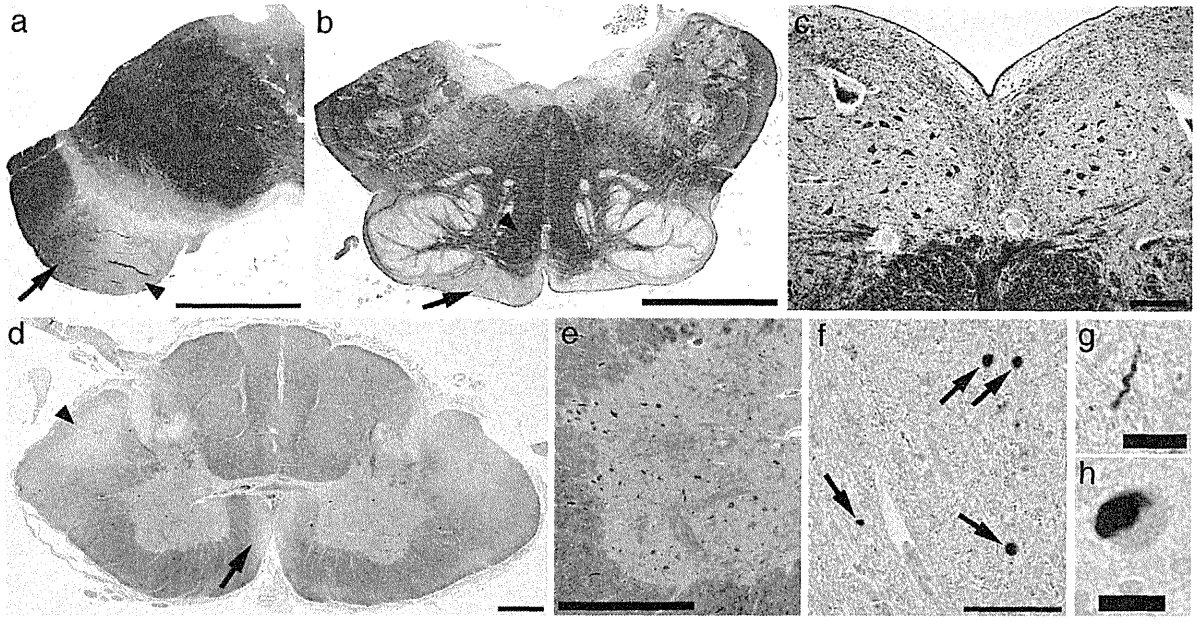

Fig. 2. (case 10) a Myelin pallor was demonstrated in the middle third of the cerebral peduncle corresponding to the corticospinal tract (CST) (arrow), although more marked degeneration was observed in the medial third of the cerebral peduncle corresponding to the frontopontine tract (arrowhead). b Atrophy and myelin pallor were demonstrated bilaterally in the pyramid of the medulla oblongata (arrow). In contrast, myelin was well preserved in the medial lemniscus (arrowhead). c Neurons were preserved in the hypoglossal nucleus. d Myelin pallor was demonstrated in the lateral (arrowhead) and anterior (arrow) CST of the cervical cord. e The cervical cord anterior horn cells were preserved. f TDP-43 positive round structures were demonstrated in the neuropil of the cervical cord anterior horn. g, h A DN (g) and a neuronal cytoplasmic inclusion (NCI) (h) were observed in the cervical cord anterior horn. a–e Klüver–Barrera stain, f–h anti-TDP43. *Scale bars* a, b 5 mm, c 200 μm, d, e 1 mm, f 50 μm, g, h 20 μm.

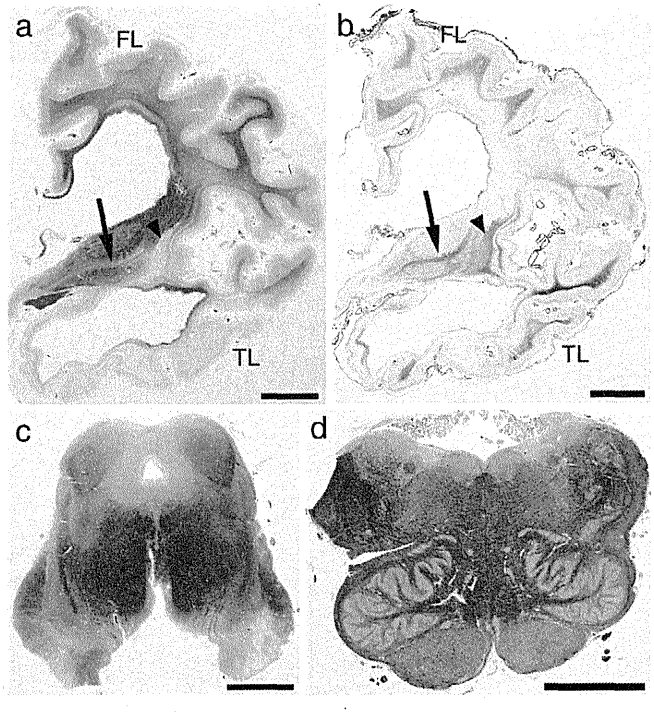

Fig. 3. a and b are serial sections of case 8. a The left hemispheric section at the plane of the posterior limb of the internal capsule showed the atrophy of the frontal lobe (FL), temporal lobe (TL) and putamen (arrowhead). The lateral ventricle was markedly enlarged. Myelin pallor was demonstrated in the posterior limb of the internal capsule (arrow), and white matter of the FL and TL. Betz cells were not identified in this section. b Gliosis was observed in the posterior limb of the internal capsule (arrow), putamen (arrowhead), and white matter of the FL and TL. c Myelin pallor was evident in the middle third of cerebral peduncle in case 6. d Myelin pallor without atrophy was observed bilaterally in the pyramid of the medulla oblongata in case 4. a, c, d Klüver–Barrera stain, b Holzer stain. *Scale bars* a, b 1 cm, c, d 5 mm.

predominantly on the right side. In case 11, the degree of the degeneration of the cerebrum could not be evaluated microscopically because there were severe hypoxic changes throughout the cerebrum. In all other cases, neuronal loss and gliosis in the frontotemporal cortices were noted only where macroscopic atrophy was demonstrated. In the PMC, neuronal loss in the superficial layer was demonstrated only in case 13, while Betz cells were sparse and showed atrophy in cases 13–15. The CST was involved in the spinal cord in all five cases in which the spinal cord could be examined. Degeneration of the pyramid of the medulla oblongata was limited to show myelin pallor without atrophy (mild, 1+) in cases 11 and 13–16, and was not apparent in case 12. The CST was spared in the cerebral peduncle in cases 11–15. In all cases, the CST was involved neither in the internal capsule nor subcortical white matter of the PMC, although the subcortical white matter of the PMC could not be evaluated in case 16 because diffuse leukoaraiosis related to the hyalinosis of the vessel walls was present. There were evident LMN loss and Bunina bodies in the hypoglossal nucleus and spinal cord in all cases.

TDP-43 immunohistochemistry showed type 2 histology in five of the six cases (cases 11–13, 15 and 16), and type 3 histology in case 14, in which a small number of NIIs was demonstrated in the frontal cortex. In the PMC, NCIs were seen in cases 11 and 14, although the PMC of cases 13 and 15 could not be evaluated by immunohistochemistry. There were no NCIs in the Betz cells in any case. In all six cases, NCIs were demonstrated in the LMNs of the brainstem and/or spinal cord, and GCIs were variably seen in the gray and white matter including the CST. In the spinal cord anterior horn, GCIs were demonstrated in cases 11 and 15, and DNs were seen in case 11. Only in case 13, TDP-43-positive round structures were observed in the neuropil of the anterior horn.

3.4. Comparison between FTLD-MND-P and FTLD-MND-A cases

Comparison of the clinicopathological findings between FTLD-MND-P and FTLD-MND-A is shown in Table 5. The mean disease duration of

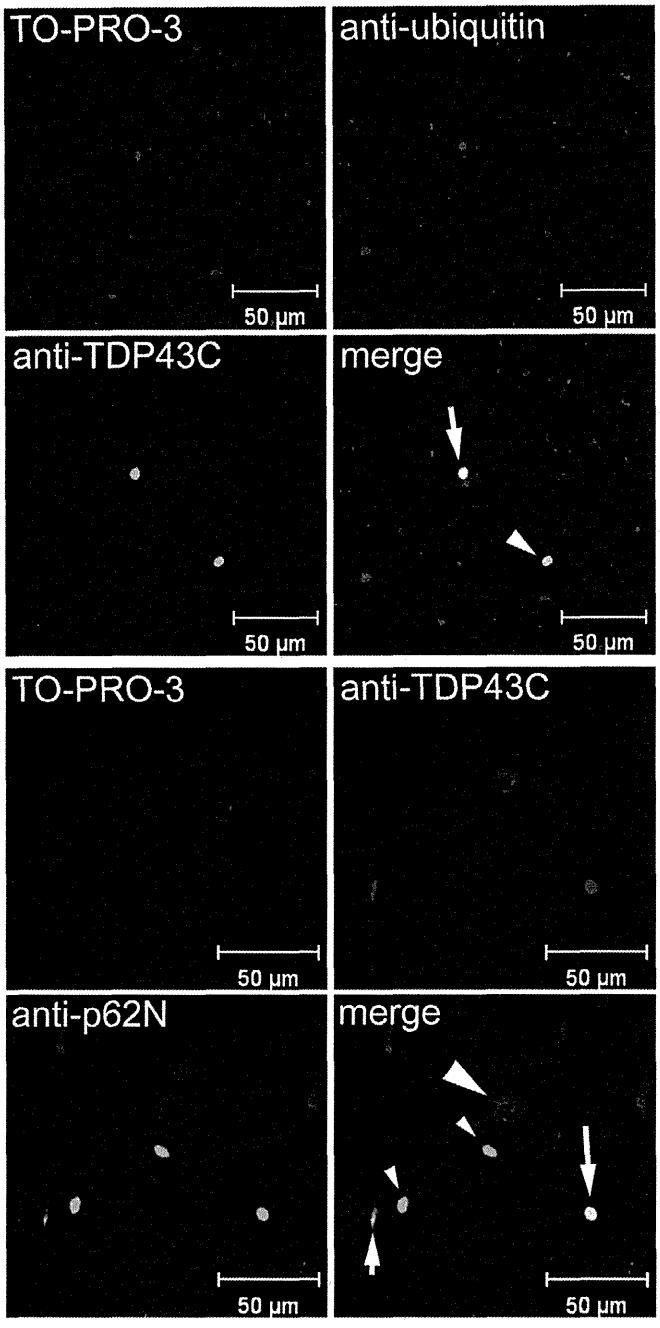

Fig. 4. The cervical cord anterior horn of case 10 was investigated. Nuclei were stained with TO-PRO-3 (Invitrogen, Tokyo, Japan), producing a blue color. Confocal immunofluorescence of ubiquitin and TDP-43C [405–414] showed colocalization in an NCI (arrow) and a round structure in the neuropil (arrowhead). Confocal immunofluorescence of TDP-43C [405–414] and p62N demonstrated colocalization in a DN (short arrow) and a round structure in the neuropil (long arrow). The small arrowheads show NCIs immunoreactive only for p62N, and the large arrowhead an NCI immunoreactive for only TDP-43.

FTLD-MND-P (12 years) was much longer than that of FTLD-MND-A (3.3 years). The CST degeneration was severe inFTLD-MND-P cases, for instance, the pyramid of the medulla oblongata showed atrophy in seven of the ten FTLD-MND-P cases while FTLD-MND-A cases did not show atrophy of the pyramid. In addition, CST degeneration was seen in the cerebral peduncle in six of the ten FTLD-MND-P cases, whereas it was demonstrated only in one of the six FTLD-MND-A cases. Small brain weight and severe CST degeneration in FTLD-MND-P cases may be partly explained by the prolonged disease duration.

4. Discussion

In our FTLD-MND-P cases, UMN signs were observed in six of ten cases, whereas LMN signs were not seen in any case. The relatively prolonged disease duration of FTLD-MND-P cases may be related to the lack of LMN signs. Histopathologically, there was no obvious LMN loss or Bunina bodies in any case. These clinical and histopathological findings are distinct from those of FTLD-MND-A. Considering the type of TDP-43 histology in the cerebral cortex, however, type 2 or 3 histology was demonstrated in the cerebral cortex of some FTLD-MND-P cases; therefore, it may be difficult to exclude the possibility that these FTLD-MND-P cases are actually an atypical form of FTLD-MND-A. The limitation of this study is that the spinal cord tissue was not available from six of ten FTLD-MND-P cases. In our institution, however, there were no FTLD-MND-A cases showing spinal cord anterior horn degeneration without hypoglossal nucleus involvement, and this finding in FTLD-MND-A has also been described by other investigators [27]. The spinal cord anterior horn should be fully examined in a larger number of FTLD-TDP cases in the future study.

Among the 29 FTLD-TDP cases examined in our institution, the LMNs were involved in all cases showing type 2 histology except for one case (case 2), whereas they were involved only in one (case 14) of the cases showing type 3 histology and never affected in cases showing type 1 histology. Based on this finding, the increases in disease duration from type 2 to 3, and to 1 may be explained by the degree of LMN involvement in each subtype.

The CST is involved in various brain diseases such as cerebrovascular diseases or degenerative diseases, and the latter is not limited to MND [17–19,28–32]. In contrast to the cerebrovascular diseases, CST degeneration in the degenerative diseases is often more severe at lower than at higher levels, and is thought to arise from axonopathy with peripheral "dying back" [29,33,34]. As a result of slow and gradual involvement of the motor cortex in degenerative diseases, the axon most distant from the motor cortex may be first affected. In our FTLD-MND-P cases, the CST was involved in the spinal cord and medulla oblongata in all cases in which these structures could be examined, but the CST in the midbrain, internal capsule, and subcortical white matter of the PMC was spared in some cases. CST degeneration in FTLD-MND-P appeared to be related to peripheral dying back as seen in other degenerative diseases.

To date, cases of FTLD with ubiquitin-positive, tau-negative inclusions (previously called as FTLD-U) showing CST degeneration but lacking both LMN loss and Bunina bodies have been reported not only from our institution but from other institution in Japan [28,31]. The disease duration of these two cases was 9 years [28] and 11 years [31], respectively. TDP-43 immunohistochemistry is needed to determine whether these cases are FTLD-TDP.

In this study, we showed for the first time the accumulation of TDP-43 in the spinal cord of cases showing type 1 histology, which also showed colocalization with ubiquitin and p62. Interestingly, this TDP-43 rarely accumulated in the cytoplasm of neurons as seen in the cerebral cortex showing type 1 histology, and manifested as round structures in the neuropil of the anterior horn. We speculate that one part of the DNs is not seen because these round structures are more frequently observed than typical vermiform DNs. The morphology suggested swollen processes like spheroids, but the site of accumulation is unclear at present. Since LMN loss was not apparent in the spinal cord of cases showing type 1 histology, these round structures do not appear to result in neuronal loss in the anterior horn.

In conclusion, our FTLD-MND-P cases showed characteristic clinicopathological features distinct from those of FTLD-MND-A. Type 1 of TDP-43 histology in FTLD-MND-P cases suggests that the underlying disease process differs from that of FTLD-MND-A, while cases showing type 2 or 3 histology might be the atypical form of FTLD-MND-A.

Table 5
Comparison between FTLD-MND-P and FTLD-MND-A cases in our institution.

	FTLD-MND-P ($N=10$)	FTLD-MND-A ($N=6$)
Clinical features		
Age of onset	54 years (range 48–75 years)	55 years (range 39–64 years)
Mean disease duration	12 years (range 2.7–21)	3.3 years (range 1.7–6)
LMN signs	absent	present
UMN signs	present in 6 of 10 cases	present in 5 of 6 cases
Pathological features		
Mean brain weight	946 g (range 640–1350)	1232 g (range 1120–1340)
CST degeneration	severe	mild
LMN loss	absent (or minimal)	evident
TDP-43 positive NCIs in LMNs	absent (or rare)	present
Bunina bodies	absent	present
Common TDP-43 histology	type 1	type 2

LMN lower motor neuron, *UMN* upper motor neuron, *CST* corticospinal tract, *NCI* neuronal cytoplasmic inclusion.

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3）認知症疾患モデル「TDP-43脳脊髄異常蓄積マウス」の開発

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key words　TAR-DNA binding protein 43 (TDP-43), transgenic mouse model, frontotemporal lobar degeneration (FTLD), amyotrophic lateral sclerosis (ALS)

要　旨

タウ陰性前頭側頭葉変性症や筋萎縮性側索硬化症ではTDP-43異常蓄積が病理プロセスの上流に位置すると考えられている．マウスにTDP-43遺伝子を導入・過剰発現させてこれら疾患のモデルを作製することが試みられているが，これまでに得られたTDP-43トランスジェニックマウスはヒト疾患の病態を十分再現できているとは言い難い．TDP-43の神経細胞における過剰発現は神経細胞の変性を引き起こし，症状として運動機能障害を呈するが，このような効果はTDP-43遺伝子変異の有無にかかわりなく生じる．また，症状発現と中枢神経系におけるTDP-43異常蓄積の多寡との関係も明らかではない．TDP-43異常が疾患を引き起こす機序がloss-of-functionであるのか，あるいはtoxic gain-of-functionであるのかなども含め，今後に残された課題は多い．

動　向

2006年秋に，タウ陰性ユビキチン陽性封入体を伴う前頭側頭葉変性症（frontotemporal lobar degeneration: FTLD）のユビキチン陽性封入体の主要構成蛋白質がTAR DNA-binding protein 43 (TDP-43) であることが明らかになった際，同時に，筋萎縮性側索硬化症（amyotrophic lateral sclerosis: ALS）にもTDP-43異常蓄積が生じていることが報告された．病理組織学的には，ALS脊髄運動ニューロンに認められるユビキチン陽性のスケイン様封入体や円形封入体がTDP-43陽性を示す．これらの封入体に蓄積したTDP-43は，凝集・不溶化，異常リン酸化，C末断片形成など，TDP-43蓄積によるFTLD（FTLD-TDP）と同様の生化学的修飾を受けている．こうしてFTLD-TDPとALSは，TDP-43蓄積症（proteinopathy）という単一の病態が，認知症と運動障害という，それぞれ異なる現れ方をしたものではないかと考えられるようになった．

もしそうであればTDP-43 proteinopathyを再現するモデル動物の作製は，これら疾患の治療法開発の鍵となる．家族性ALSの一部がTDP-43遺伝子（*TARDBP*）変異により生じることが報告されて，TDP-43が病因蛋白質であるという説はさらに確かなものになった．これまでにTDP-43 proteinopathyモデル動物は，ショウジョウバエからサルに至るまで様々な動物種で作製が試みられ，それぞれに成果を挙げているが，Aβやタウのような確立されたモデルはまだ得られていないように思われる．本稿では特にマウスモデルに焦

点を絞って現状を概説するが，執筆時点までに報告された10を超える多様なトランスジェニック(Tg) マウス系統での知見には，系統間あるいは研究者間で一致していない部分が多く存在する．遺伝子導入によるTDP-43の過剰発現は，多くの場合，マウスに運動機能障害を起こすが，TDP-43異常蓄積という点ではこれらのマウスの所見は一貫しておらず，かつ厳密な意味でヒト疾患と同様のTDP-43異常蓄積を再現しているとは言い難い．

A. TDP-43の異常

FTLDやALS患者脳脊髄におけるTDP-43異常蓄積にはいくつかの特徴がある．TDP-43は不均一核内リボ蛋白質ファミリーに属する蛋白質で，N末側に核移行シグナル（nuclear localization signal: NLS）を有し（アミノ酸残基78～84番目），正常細胞では主として核に局在する．しかしTDP-43 proteinopathyの脳脊髄において細胞質に封入体を形成した細胞の核ではTDP-43は著しく減少し，病理組織標本の免疫組織化学染色ではほとんど検出されなくなる．TDP-43の生理機能は十分明らかにされてはいないが（転写やスプライシング，翻訳などのプロセスにおける調節に関わると考えられている），異常細胞における核からの消失という所見は，TDP-43の機能が失われることで細胞が変性に陥るとするloss-of-function説の根拠となっている．

また異常蓄積したTDP-43はsarkosylなどの界面活性剤に対して不溶性になるとともに，異常リン酸化，ユビキチン化といった修飾を受ける．さらにN末側が切断されて，分子量約18～26kDaの複数のC末側断片が形成され，このC末側断片が，不溶化して蓄積したTDP-43のかなりの割合を占める．こういった変化は，タウやαシヌクレインの異常蓄積と共通している．このように，局在も機能も大きく異なるいくつもの蛋白質が同じような変化を受けて過剰に蓄積することと神経細胞変性とが関連していることから，TDP-43の場合も異常蓄積が細胞障害性に作用すると考えるtoxic gain-of-function説もある．

いずれにせよ，こういった疾患脳脊髄で認められる変化は，モデル作製の際のツールとなったり，あるいは作られたモデルがどこまでヒト病変を再現しているかという検証に用いられたりするという点で，疾患を解決するための研究の基盤となる情報である．

B. TDP-43遺伝子(*TARDBP*)変異

家族性に発症するALSの中にALS-10と呼ばれていた群があり，2008年に*TARDBP*変異が原因であることが明らかになった．*TARDBP*変異によるALSは家族性ALSの数%，孤発性ALSの1%前後を占めるに過ぎないが，孤発性ALSや*C9orf72*のリピート伸長によるALSとは，TDP-43の異常蓄積という点で共通の病理変化を持つ．言い換えると，ALSの大半は*TARDBP*変異による家族性ALSと同じ病態にもとづき発病している可能性が高い．ALSの原因となる*TARDBP*変異は多数見出されているが，そのほとんどがTDP-43のC末側部分に存在する．ただ，*TARDBP*変異がどのような機序で発病に結びつくかは不明である．この点はTgマウスの作製，解析によっても明確な答は得られていない．また*TARDBP*変異がFTDのみという病型をとることは稀で，ほとんどの家系はALSの病型をとるが，この理由もわかっていない．ただ，*TARDBP*変異の発見はTDP-43異常が病因であるという考え方の重要な根拠となり，さらに，タウやAβ前駆体蛋白質の病因遺伝子変異の導入が疾患モデルマウス開発に結びついたこれまでの経験から，TDP-43 proteinopathyのモデルとしてALSの原因となる変

異*TARDBP*を過剰発現させたTgマウスが次々と作製されることになった．

C. 通常のTDP-43-Tgマウス

本稿執筆時点までに，主要なジャーナルだけで10を超すTgマウス作製の論文が報告されている（表1）[1-11]．多くはヒトTDP-43 cDNAを，中枢神経疾患のTgマウス作製に頻用されるプロモーター（プリオン，Thy1.2，CaMKIIなどのプロモーター）を用いて過剰発現させたもので，野生型TDP-43に加え，A315T，M337Vといったalsの変異を持つ導入遺伝子が用いられている．得られた系統の表現型は，胎生致死から，ほぼ正常に発育～加齢するものまで様々で，そういった系統差の少なくとも一部は導入遺伝子の発現量と関係していると推測されている．これらの研究では，生後一定期間以上生存し，さらに交配による維持が可能な系統を中心に解析が行われているが，そのような系統の多くで生後早期からの運動機能障害と寿命短縮が報告されている．運動機能障害とともに学習障害が確認された系統もある．導入遺

表1　TDP-43トランスジェニックマウス

筆頭著者	誌名	発現TDP-43遺伝子	表現型
Wegorzewska I	PNAS 2009 [1]	A315T	前頭葉第V層と脊髄前角の神経細胞にユビキチン（+）封入体（核のTDP-43減少を伴うが，封入体はTDP-43陰性）．3～4カ月齢で歩行障害．短命
Wils H	PNAS 2010 [2]	wild	hTDP-43発現量に応じた運動ニューロンの変性（前頭葉第V層と脊髄）・四肢麻痺，稀にリン酸化TDP43（+）封入体（核内・細胞質，核のTDP-43減少を伴う），TDP-43C末断片形成（～25kDa，核Tx不溶/Sarkosyl可溶画分），homoとheteroとの比較ではhomoに強い変化
Tsai KJ	J Exp Med 2010 [3]	wild（mouse）	学習障害，進行性の運動機能障害，海馬萎縮（神経細胞のアポトーシス），加齢に伴うTDP-43（+）ユビキチン（+）細胞質封入体出現（核のTDP-43減少を伴う）
Stallings NR	Neurobiol Dis 2010 [4]	wild, A315T	変異TDP-43の場合は脊髄でのTDP-43発現量に応じて誕生早期からの運動障害．A315Tの系統の中に発育後の麻痺，核からのTDP-43減少，TDP-43断片形成，ユビキチン陽性細胞質封入体とグリオーシス．Wild TDP-43では（発現量が高くても）症状が出ず
Xu YF	J Neurosci 2010 [5]	wild	hTDP-43の発現量に応じたmTDP-43の減少．中程度のhTDP-43発現の場合：C末断片形成，細胞質・核のユビキチン増加，稀にリン酸化TDP-43陽性核内・細胞質封入体，ミトコンドリア異常蓄積，歩行障害，短命
Shan X	PNAS 2010 [6]	wild	発育不良，短命，リン酸化TDP-43（+）細胞質封入体なし，TDP-43/FUS（+）核内封入体形成，細胞質へのミトコンドリア蓄積（封入体形成）と軸索でのミトコンドリア欠損
Igaz LM	J Clin Invest 2011 [7]	wild, ΔNLS (tetOff)	神経細胞変性・錐体路変性，痙性，mTDP-43発現低下，ごく少数のリン酸化TDP-43（+）ユビキチン（+）凝集体（RIPA不溶画分に出現）
Swarup V	Brain 2011 [8]	wild, G348C, A315T	cDNAではなくhTDP-43遺伝子断片を発現．老齢でTDP-43が細胞質にも出現・封入体形成，一部ユビキチン化・不溶化・断片形成（リン酸化は不明）．加齢とともに運動機能障害，学習障害
Xu YF	Mol Neurodegener 2011 [9]	wild, M337V	核内・細胞質にリン酸化TDP-43（+）凝集体が高頻度に出現，～25/35kDa断片形成，ユビキチン増加を伴う．mTDP-43は減少．細胞質のミトコンドリア凝集，グリオーシス．歩行障害，短命
Caccamo A	Am J Pathol 2012 [10]	CTF（25kDa）	可溶性画分のTDP-43 C末断片（25kDa）増加に伴う学習障害（神経変性や封入体形成とは無関係に生じた）
Cannon A	Acta Neuropathol 2012 [11]	wild (tetOff)	離乳後On：細胞質でのリン酸化TDP-43の顆粒状凝集（ユビキチン陽性），神経細胞変性．発生段階からOnの場合は，これらにミトコンドリア異常と短命が加わる