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Original Article

Pseudopolyneuritic form of ALS revisited: Clinical and pathological heterogeneity

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Pseudopolyneuritic form of ALS is a subtype of ALS characterized by distal weakness of the unilateral lower limb and absence of Achilles tendon reflex (ATR) at disease onset. Recognition of this form of ALS is important for clinicians because the combination of distal weakness of the lower limb and absence of ATR usually suggests peripheral neuropathy. We reviewed the clinical records of 42 autopsy-proven sporadic ALS cases and found three cases that showed onset of weakness of the unilateral lower limb with distal dominance and absence of ATR. The disease duration in the three cases was 2, 3 and 19 years, respectively. The clinical features of the patient with a course of 19 years had been restricted to lower motor neuron signs. Histopathologically, consistent findings in the three cases were severe motor neuron loss throughout the whole spinal cord, with relative preservation of the hypoglossal nucleus. Reflecting this finding, TDP-43-positive neuronal cytoplasmic inclusions in the spinal cord were sparse in two cases, and absent in a third. In the patient showing a clinical course of 19 years, mild corticospinal tract degeneration appeared to correspond to the absence of upper motor neuron signs and prolonged disease duration. In this case only, Bunina bodies were not demonstrated. In this study, we clarified the clinical and pathological heterogeneity of this form of ALS.

Key words: Achilles tendon reflex, amyotrophic lateral sclerosis, heterogeneity, pseudopolyneuritic form, TDP-43.

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INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder that affects both upper motor neurons (UMNs) and lower motor neurons (LMNs). Typically, UMN and LMN signs coexist in the affected limbs at onset, and the former manifests as hyper-reflexia.[1] One of the recent reports has described that upper-limb onset was seen in ≈ 48% of patients, and lower-limb onset in ≈ 24%.[1] At autopsy, the average loss of LMNs is ≈ 50%.[2,3]

The pseudopolyneuritic form of ALS is a subtype of ALS characterized by distal weakness of the unilateral lower limb and absence of Achilles tendon reflex (ATR) at disease onset.[4–6] The patellar and upper limbs tendon reflexes may show hyper-reflexia.[4,6] The survival time with this form has been reported to range from 30 to 69 months,[4,5,7–10] and the frequency is from 1% to 17.5%.[5,7,8,10]

Histopathologically, preferential cellular degeneration of the lumbar cord was described originally,[4] and later severe LMN loss throughout the whole spinal cord[11] and depletion of the small neurons in the intermediate zone of the anterior horn of the lumbar cord[12] were reported. However, to date information regarding the clinical and histopathological findings of this disease has been limited. The purpose of our study is to describe the clinicopathological findings of three cases of the pseudopolyneuritic form of ALS including one case that showed only LMN signs and a markedly prolonged disease duration.

MATERIALS AND METHODS

Subjects

We reviewed the clinical records of 42 autopsy-proven sporadic ALS cases including details of the symptoms at onset

and total clinical course, from the institutional collections at Tokyo Institute of Psychiatry in Japan. There were 19 men and 23 women. The mean age at onset was 62.3 years (range: 33–83 years). The mean disease duration of the cases without artificial respiratory support was 30.8 months (range: 2–228 months). There were 15 cases showing upper-limb onset and nine cases of lower-limb onset. In the cases showing lower-limb onset, deep tendon reflex was recorded in seven cases. Among these cases, we encountered three cases that showed onset of unilateral lower-limb weakness with distal dominance and absence of ATR. One of these cases (case 3) was previously reported.[13] For comparison, we examined specimens from 11 cases of upper-limb onset ALS and four ALS cases showing lower-limb onset with increased ATR as controls.

Conventional neuropathology and assessment of LMN loss

Brain tissue samples from all subjects were fixed post mortem with 10% formalin and embedded in parafin. Sections (10 μm thick) were prepared from the frontal, temporal, parietal, occipital, insular and cingulate cortices, hippocampus, amygdala, basal ganglia, midbrain, pons, medulla oblongata, cerebellum and spinal cord, including the cervical, thoracic, lumbar and sacral cords. These sections were stained by HE, KB and Holzer methods. The degree of LMN loss in the hypoglossal nucleus and the spinal cord was graded as mild, moderate or severe.

Immunohistochemistry and assessment of TDP-43 pathology

Antibodies used in this study are shown in Table 1. Sections from representative regions of the cerebrum, brainstem, and cervical, thoracic, lumbar and sacral cords were examined using antibodies to ubiquitin and phosphorylated TDP-43 (pS409/410).[14] In case 3, TDP-43 immunoreactivity was examined further using other anti-phosphorylated TDP-43 antibodies (pS403/404)[14] and two kinds of phosphorylation-independent antibodies (anti-TDP-43C antibodies [405–414][14] and commercially available antibodies). Hippocampal dentate granular cells of case 2 were also examined with these three anti-TDP-43 antibodies, and were further examined with anti-fused in sarcoma (FUS),[15] anti-AT8, and anti-α-synuclein antibodies. In case 3, cystatin C immunoreactivity was examined in the brainstem and spinal cord, and axons in the corticospinal tract (CST) of the spinal cord were evaluated using anti-neurofilament antibodies. The severity of TDP-43 immunoreactive pathological changes in each topographical brain area was rated as: 0 = absent; 1 = rare to mild; 2 = moderate to severe.

RESULTS

Case reports

None of the three cases had any history of ALS-like disorder in their families. Case 3 is briefly described because this case was reported previously.[13] The clinicopathological findings of the three cases are summarized in Tables 2 and 3. Distribution of TDP-43-positive inclusions is shown in Table 4.

Case 1

Clinical course. A 61-year-old Japanese man developed right dropped foot followed by left dropped foot approximately 3 months later, and presented with steppage gait. He needed a cane while walking, and consulted the Department of Neurology at a general hospital 10 months after onset. Neurological examination demonstrated muscle weakness of the distal part of the lower limbs and absence of ATR. Other tendon reflexes were within normal limits. Fasciculation was not apparent. Nerve conduction study demonstrated that motor conduction velocity was within normal limits. Needle electromyogram demonstrated neurogenic changes. Thereafter, muscle weakness of the lower limbs progressed, and he became unable to

Table 1 Antibodies used in this study

Antibody	Type	Source	Dilution
Anti-ubiquitin	Rabbit polyclonal	Dako, Glostrup, Denmark	1:2000
Phosphorylation-independent anti-TDP-43			
Anti-TDP-43	Rabbit polyclonal	ProteinTech, Chikago, IL, USA	1:1000
Anti-TDP-43C [405–414]	Rabbit polyclonal	Made by Hasegawa *et al.*[14]	1:1000
Phosphorylation-dependent anti-TDP-43			
pS409/410	Rabbit serum	Made by Hasegawa *et al.*[14]	1:1000
pS403/404	Rabbit serum	Made by Hasegawa *et al.*[14]	1:1000
Anti-FUS	Rabbit polyclonal	Sigma, St. Louis, MO, USA	1:500
Anti-tau (AT8)	Mouse monoclonal	Innogenetics, Gent, Belgium	1:100
Anti-α-synuclein (Pα#64)	Mouse monoclonal	Wako Chemical, Osaka, Japan	1:3000
Anti-cyctatin C	Rabbit polyclonal	Dako, Glostrup, Denmark	1:1000
Anti-neurofilament (SMI 31)	Mouse monoclonal	Sternberger, Lutherville, MD, USA	1:1000

Table 2 Clinical features of the three cases

	Case 1	Case 2	Case 3
Age at onset (years)	61	59	42
Gender	Male	Female	Female
Disease duration (months)	25	39	228
Site of initial symptom	Distal part of the right lower limb	Left lower limb	Distal part of the left lower limb
Deep tendon reflex at onset			
Upper limb (right/left)	Normal/normal	Increased/increased	Normal/normal
Patellar (right/left)	Normal/normal	Increased/increased	Normal/decreased
Achilles (right/left)	Absent/absent	Absent/absent	Decreased/absent
Babinski's sign (right/left)	Negative/negative	Positive/positive	Negative/negative
Upper motor neuron sign (throughout the course)	Present	Present	Absent
Bulbar symptom (initially/eventually)	Absent/present	Absent/present	Absent/present
Clinical diagnosis	Pseudopolyneuritic form of ALS	Pseudopolyneuritic form of ALS	Spinal progressive muscular atrophy

Table 3 Neuropathological findings of the three cases

	Case 1	Case 2	Case 3
Brain weight (g)	1410 (after fixation)	1310 (before fixation)	1230 (before fixation)
Lower motor neuron loss			
Hypoglossal nucleus	Moderate	Moderate	Moderate
Spinal cord	Severe at all levels	Severe at all levels	Severe at all levels
Distribution of Bunina bodies	Trigeminal nucleus, hypoglossal nucleus, anterior horn (C6, C8, L3, S2)	Hypoglossal nucleus	–
Corticospinal tract degeneration			
Posterior limb of internal capsule	–	–	–
Midbrain	+	–	–
Medulla oblongata	+	+	–
Cervical cord	+	+	–
Thoracic cord	+	+	–
Lumbar cord	+	+	+

walk at age 62. Subsequently, he developed muscle weakness of the bilateral upper limbs and dysarthria, and was admitted to our hospital 1 year and 10 months after onset. Neurological examination demonstrated mild facial palsy. Tongue atrophy or fasciculation was not apparent. Muscle weakness was also demonstrated in all four limbs, and the distal part of the lower limbs showed complete paralysis. Muscle atrophy was prominent in the bilateral tibialis anterior muscles. ATR was absent, and the patellar tendon reflex was within normal limits on the right side, and was increased on the left side. The bilateral upper limbs showed hyper-reflexia. Muscle biopsy showed neurogenic changes. A clinical diagnosis of the pseudopolyneuritic form of ALS was made. The patient refused artificial respiratory support and died of respiratory failure.

Neuropathological findigs. Microscopically, the lateral part of the anterior horn was atrophic in the lumbar and cervical cord (Fig. 1a,d). Severe LMN loss was demonstrated throughout the whole spinal cord (Fig. 1a,c,d). A few neurons were demonstrated in the intermediate zone in the lumbar cord. Moderate neuronal loss was demonstrated in the hypoglossal nucleus (Fig. 1e) and trigeminal motor nucleus. Myelin pallor in the CST was demonstrated in the whole spinal cord (Fig. 1a,b), medulla oblongata and midbrain, but was not obvious in the internal capsule. In the anterior funiculus of the thoracic cord, myelin pallor was demonstrated beyond the CST[16] (Fig. 1b). Neuronal loss was not apparent in the cerebrum, although there was sparse accumulation of lipid-laden macrophages in the shape of a Betz cell in the precentral gyrus. Bunina bodies were demonstrated in the LMNs. Only one ubiquitin-positive skein-like neuronal cytoplasmic inclusion (NCI) was found in the anterior horn of the cervical cord. TDP-43-positive NCIs were demonstrated in the entorhinal, transentorhinal and occipitotemporal cortices, amygdala, globus pallidus, inferior olivary nucleus, reticular formation of the medulla, and anterior horn of the thoracic, lumbar and sacral cords. Glial cytoplasmic inclusions (GCIs) were distributed more frequently and extensively, that is, in the frontal, temporal, and parietal lobe, amygdala, globus pallidus, thalamus, cerebral peduncle, trigeminal motor nucleus, pontine nucleus, hypoglossal nucleus, inferior olivary nucleus, reticular formation of the medulla,

Table 4 Distribution and severity of TDP-43 pathology across scanned central nervous system regions

	Case 1		Case 2		Case 3	
	NCI	GCI	NCI	GCI	NCI	GCI
Cerebrum						
Cingulate gyrus cortex	0	0	0	0	0	0
Cingulate white matter		0		0		0
Frontal cortex	0	1	0	1	0	0
Frontal white matter		1		0		0
Motor cortex	0	2	0	1	0	0
Motor white matter		2		0		0
Anterior parietal cortex	0	1	0	0	0	0
Anterior parietal white matter		1		0		0
Amygdala	1	2	0	0	0	0
Dentate gyrus	0	0	0	0	0	0
CA/subiculum	0	0	0	0	0	0
Entorhinal cortex	1	1	0	0	0	0
Entorhinal white matter		1		0		0
Insular cortex	0	1	0	0	0	0
Insular white matter		0		0		0
Temporal cortex	1	1	0	0	0	0
Temporal white matter		1		0		0
Striatum	0	0	0	0	0	0
Globus pallidus	1	1	0	0	0	0
Thalamus	0	1	0	0	0	0
Posterior limb of internal capsule		0		0		0
Midbrain						
Reticular formation	0	0	NA		0	0
Red nucleus	0	0	0	0	0	0
Substantia nigra	0	0	0	0	0	0
Cerebral peduncle		1		0		0
Pons						
Trigeminal nucleus	0	2	1	0	NA	
Facial nucleus	NA		NA		1	0
Reticular formation	0	0	0	0	0	0
Pontine nucleus	0	1	0	1	0	0
Pramidal tract		0		0		0
Medulla oblongata						
Hypoglossal nucleus	0	1	1	1	1†	1
Inferior olivary nucleus	1	1	0	1	0‡	0
Reticular formation	1	2	1	2	0	0
Pyramid		1		1	0	0
Spinal cord						
Anterior horn	1	2	1	0	0	0
Ventral corticospinal tract		1		0		0
Lateral corticospinal tract		1		0		0

†Dystrophic neurites were also found. ‡TDP-43-positive round structures were found in the neuropil. In case 3, the distribution and severity were evaluated using anti-TDP-43C [405–414] antibody. 0, no pathology; 1, rare to mild pathology; 2, moderate to severe pathology; GCI, glial cytoplasmic inclusion; NA, tissue not available; NCI, neuronal cytoplasmic inclusion.

pyramid, and whole spinal cord. In the cerebrum, numerous GCIs were demonstrated in the precentral gyrus (Fig. 2). These were frequently seen in the deep layers of the cortex, and less distributed in the superficial layer and subcortical white matter. In the spinal cord, numerous GCIs were seen in the anterior horn of the lumbar cord (Fig. 1f).

Case 2

Clinical course. A 59-year-old Japanese woman developed weakness in the left leg. Ten months after onset, she was admitted to the Department of Neurology of a general hospital. Neurological examination showed atrophy and fasciculation of the left lower limb, especially in the distal part. ATR was absent bilaterally, whereas patellar and upper-limb tendon reflexes were increased bilaterally. A clinical diagnosis of the pseudopolyneuritic form of ALS was made. At age 62, she was emergently admitted to our hospital because of dyspnea. Neurological examination demonstrated atrophy and fasciculation of the tongue, dysarthria, dysphagia, and muscle atrophy and weakness in all four limbs. ATR was absent, and patellar and upper-limb tendon reflexes were decreased. There was neither character change nor dementia. The patient refused artificial respiratory support and died of respiratory failure approximately 18 days after admission.

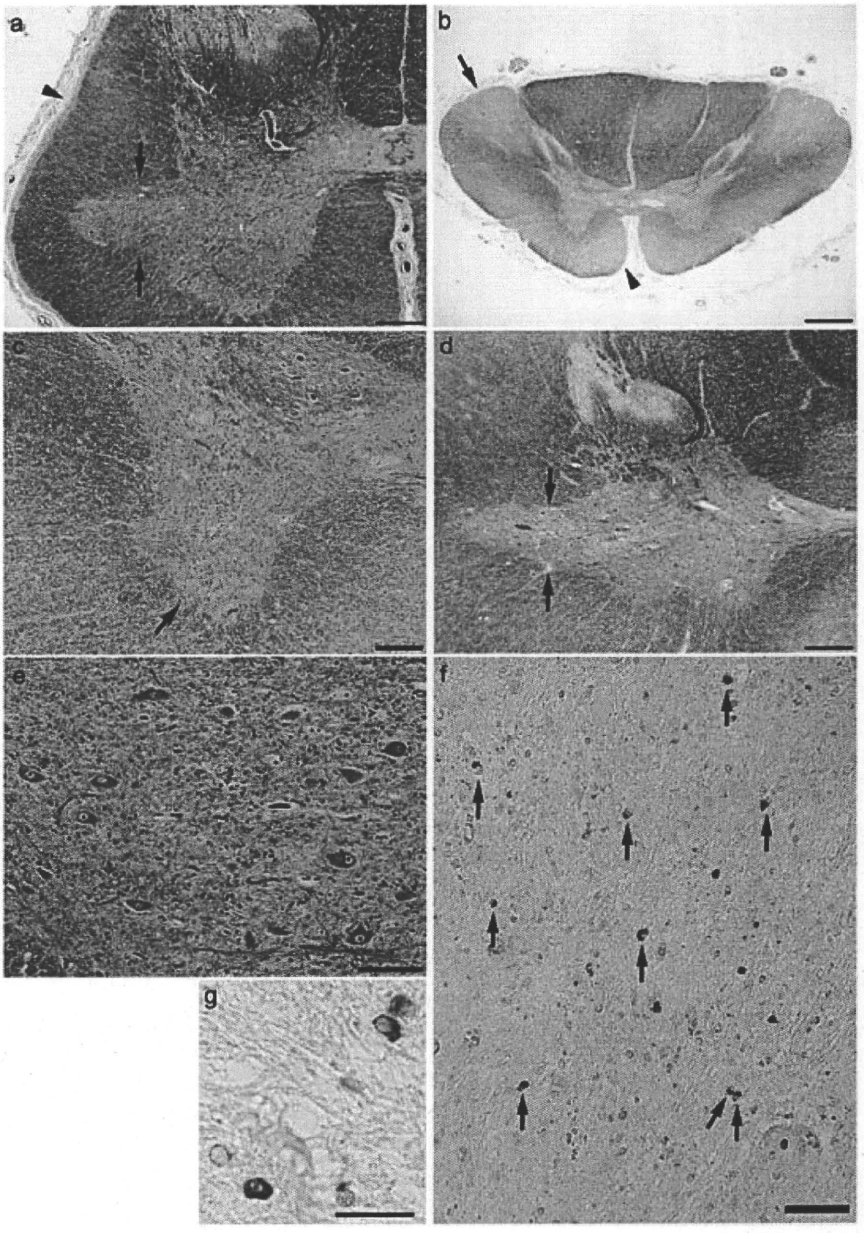

Fig. 1 Front and back diameter of the lateral part of the anterior horn was reduced in lumbar segment 5 (a, arrows). Motor neurons had almost disappeared (a). Lateral corticospinal tract (CST) showed myelin pallor and atrophy (a, arrowhead). In thoracic segment 10, myelin pallor was seen in the lateral (b, arrow) and anterior (b, arrowhead) CST with extension outside the CST in the anterior funiculus. High-power view demonstrated that motor neurons had almost disappeared (c, arrow). Front and back diameter of the lateral part of the anterior horn was reduced in cervical segment 6 (d, arrows). Motor neurons had almost disappeared (d). Moderate neuronal loss was seen in the hypoglossal nucleus (e). Glial cytoplasmic inclusions (GCIs) were frequently seen in the anterior horn of lumbar segment 5, whereas there were no neuronal cytoplasmic inclusions (NCIs) in this region (f). High-power view (g). a–e KB stain; f, g phospholylated TDP-43 (pS409/410). Scale bar a 500 μm, b 1 mm, c 200 μm, d 500 μm, e 100 μm, f 50 μm, g 20 μm.

Neuropathological findigs. Microscopically, the lateral part of the anterior horn of the lumbar cord was atrophic. Severe LMN loss was demonstrated throughout the whole spinal cord. In the anterior horn of the lumbar cord, a few neurons were demonstrated in the medial motor nucleus and intermediate zone, whereas the lateral motor nucleus showed complete neuronal loss. Moderate neuronal loss was demonstrated in the hypoglossal nucleus and trigeminal motor nucleus. Myelin pallor in the CST was demonstrated in the whole spinal cord and medulla oblongata, but was not obvious in the midbrain and internal capsule. Bunina bodies were seen in the hypoglossal nucleus. There was sparse accumulation of lipid-laden macrophages in the shape of a Betz cell in the precentral gyrus. Neuronal loss was evident in the basolateral area of the amygdala, and substantia nigra. Ubiquitin-immunoreactive NCIs were demonstrated in the hippocampal dentate granular cells, hypoglossal nucleus, and anterior horn of the lumbar cord. They were not demonstrated in the amygdala or substantia nigra. TDP-43-positive NCIs were sparsely demonstrated in the trigeminal motor nucleus, hypoglossal nucleus, reticular formation of the medulla, and anterior horn of the lumbar cord. Unexpectedly, ubiquitin-immunoreactive NCIs in the hippocampal dentate granular cells were negative for all kinds of anti-TDP-43 antibodies. These were also negative for FUS, AT8, and α-synuclein. TDP-43-positive GCIs were frequently seen in the reticular formation of the medulla, and were sparse in the cortex of the

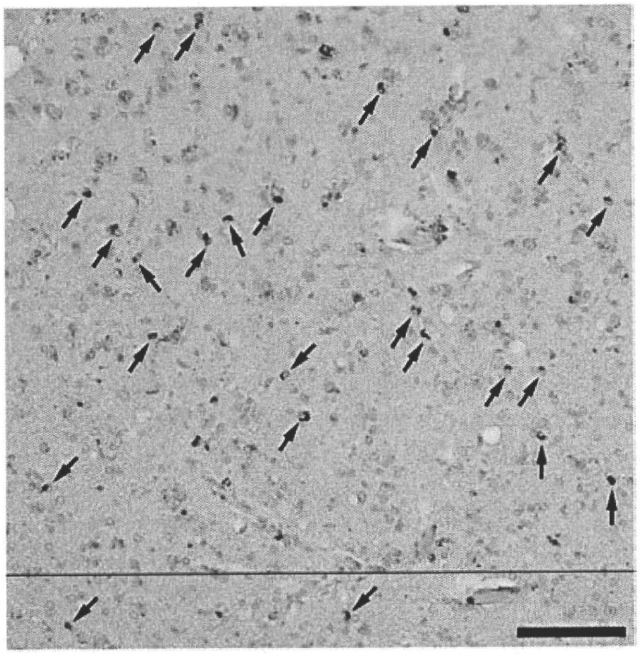

Fig. 2 A red line shows the boundary between the gray and white matter of the precentral gyrus. Immunohistochemistry using anti-phosphorylated TDP-43 (pS409/410) antibodies demonstrated that glial cytoplasmic inclusions (GCIs) (arrows) were frequently seen in the deep layer of the cortex, and less distributed in the subcortical white matter. Neuronal cytoplasmic inclusions (NCIs) were not apparent. Scale bar 50 μm.

precentral gyrus, pontine nucleus, hypoglossal nucleus, inferior olivary nucleus and pyramid, and were not demonstrated in the spinal cord.

Case 3

Clinical course. A 42-year-old Japanese woman developed muscle weakness in the distal part of the left lower limb. Neurological examination at age 46 demonstrated muscle atrophy and weakness in the left lower limb with distal predominance. The left ATR was absent, and the right ATR and left patellar tendon reflex were decreased. A diagnosis of lumbar disc herniation was made, and laminectomy of the lumbar segment 4–5 was performed. However, there was no improvement, and she began to use a cane while walking. At age 49, she developed muscle weakness of the right lower limb and the distal part of the bilateral upper limbs. She died of suffocation probably related to bulbar palsy at age 61. There were no UMN signs throughout the clinical course.

Neuropathological findings. Microscopically, severe LMN loss was demonstrated throughout the whole spinal cord. Small neurons of the intermediate zone in the lumbar cord were also lost. Moderate neuronal loss was demonstrated in the hypoglossal nucleus and facial nucleus. Bunina bodies were not demonstrated. Myelin pallor and gliosis were seen in the CST of the lumbar cord (Fig. 3a), and loss

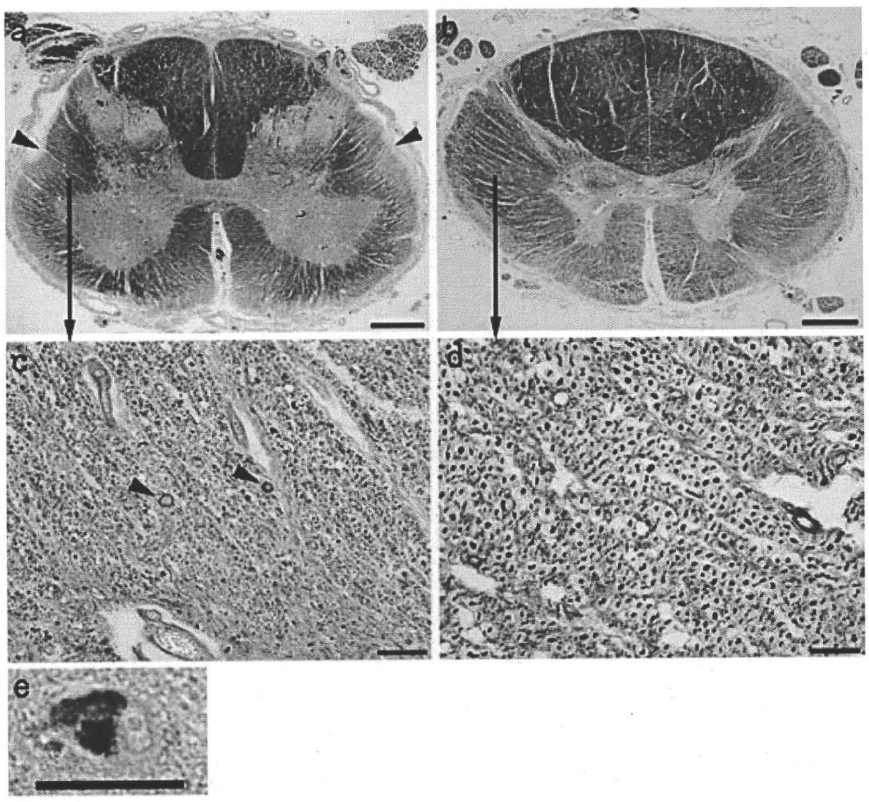

Fig. 3 Myelin pallor was demonstrated in the lateral corticospinal tract (CST) in lumbar segment 5 (a, arrowheads), but was not apparent in thoracic segment 10 (b). Axons were severely lost, and spheroids (c, arrowheads) were seen in the right lateral CST of lumbar segment 5 (c). In contrast, axons were preserved in the right lateral CST of the thoracic segment 10 (d). An neuronal cytoplasmic inclusion (NCI) was demonstrated in the hypoglossal nucleus (e). a, b KB stain; d, e neurofilament; e TDP-43C [405–414], Scale bar a, b 1 mm; c–e 50 μm.

of axons was also demonstrated by anti-neurofilament antibodies (Fig. 3c). They were not apparent in the CST of the thoracic and cervical cord (Fig. 3b,d), brainstem, and internal capsule. There was sparse accumulation of lipid-laden macrophages in the shape of a Betz cell in the pre-central gyrus. Immunohistochemical re-examination using anti-ubiquitin antibodies demonstrated a skein-like NCI in the hypoglossal nucleus, although it was not demonstrated previously.[14] Using two kinds of anti-phosphorylated TDP-43 antibodies, there were no TDP-43-positive structures, although anti-TDP-43C [405–414]) antibodies detected NCIs in the facial nucleus and hypoglossal nucleus (Fig. 3e). Dystrophic neurites and GCIs were also seen in the hypoglossal nucleus, and round structures were observed in the neuropil of the inferior olivary nucleus. NCIs in the facial nucleus and hypoglossal nucleus were also demonstrated by another phosphorylation-independent antibody. There were no TDP-43-positive structures in the cerebrum or spinal cord. There were no cystatin C immunoreactive Bunina bodies.

LMN loss of the spinal cord in the control cases

Severe LMN loss in the cervical cord was demonstrated in 13 of 15 cases, whereas that in the lumbar cord was seen only in two of four cases of lower-limb onset ALS, and in three of 11 cases of upper-limb onset ALS. In the cases showing severe LMN loss in the lumbar cord, small neurons in the intermediate zone of the anterior horn were also decreased in the lumbar cord.

DISCUSSION

The pseudopolyneuritic form of ALS is a subtype of ALS characterized by distal weakness of the unilateral lower limb and absence of ATR at disease onset.[4–6] Recognition of this form of ALS is important for clinicians because the combination of distal weakness of the lower limb and absence of ATR usually suggests peripheral neuropathy. In clinical practice, identification of the hyper-reflexia in the knees and/or upper limbs may be a key in making a diagnosis of ALS, although exceptional cases such as our case 3 may not show UMN signs.

Our three patients showed weakness of the unilateral lower limb with distal dominance and absence of ATR while other clinical features were variable among cases. At autopsy, severe LMN loss throughout the whole spinal cord seen in the three cases was concordant with the findings reported by Nishigaki *et al.*[11] However, this finding does not appear to be specific for this form because there were similar findings in five of 15 cases examined as controls. Probably, early and severe involvement of motor neurons in lumbar segment 5 and/or sacral segment 1 results in muscle weakness of the distal lower limb and absence of ATR, causing the pseudopolyneuritic form of ALS. In other words, this form does not seem to comprise a distinct pathological entity but is one phenotype based on the characteristic clinical features at onset. Among the cases showing severe LMN loss in the lumbar cord in this study, we could not find any differences between the pseudopolyneuritic form and control cases with regard to the small neurons in the intermediate zone of the anterior horn of the lumbar cord.

Reflecting the severe LMN loss throughout the whole spinal cord in our cases, TDP-43-positive NCIs in the spinal cord were sparse in cases 1 and 2, and were absent in case 3. On the other hand, various TDP-43 pathologies were also demonstrated in our three cases. In case 1, unlike the other two cases, TDP-43-positive inclusions were extensively demonstrated throughout the brain and spinal cord. In the anterior horn of the lumbar cord, numerous GCIs were demonstrated despite severe LMN loss, suggesting that glial cells were affected even after the LMNs were lost. In case 2, ubiquitinated protein in the hippocampal dentate granular cells was negative for TDP-43, FUS, AT8, and α-synuclein, therefore a yet unknown protein may have been involved in this region. In case 3, TDP-43-positive structures were demonstrated only in the brainstem, and such a limited distribution may be consistent with the findings of ALS showing prolonged disease duration.[17]

Finally, in case 3, we could not make a clinical diagnosis of ALS because there were no UMN signs throughout the clinical course. Involvement of the CST might have been detected if motor-evoked potentials had been examined.[6] At autopsy, CST degeneration was mild, and this finding appeared to correspond to the absence of UMN signs and prolonged disease duration.[17] There has been a long controversy as to whether sporadic spinal progressive muscular atrophy (SPMA) is a variant of ALS or a distinct disease. Usually, a clinical diagnosis of SPMA is made when the patient does not show UMN signs. However, at autopsy UMN pathology[17–20] and/or Bunina bodies[17] are demonstrated in some patients, suggesting that the underying pathology is similar to that of ALS in some patients with SPMA. In addition, we could not completely exclude the possibility that mutation in superoxide dismutase-1 (SOD1) was present in case 3. Although this possibility seemed unlikely based on the absence of a family history and presence of TDP-43-positive inclusions,[21,22] clinicians should know that some familial ALS cases with the SOD1 mutation show clinical features similar to those of case 3.[23–26]

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REFERENCES

1. Nalini A, Thennarasu K, Gourie-Devi M, Shenoy S, Kulshreshtha D. Clinical characteristics and survival pattern of 1,153 patients with amyotrophic lateral sclerosis: experience over 30 years from India. *J Neurol Sci* 2008; **272**: 60–70.
2. Ravits J, Laurie P, Fan Y, Moore DH. Implications of ALS focality: rostral-caudal distribution of lower motor neuron loss postmortem. *Neurology* 2007; **68**: 1576–1582.
3. Ince PG. Neuropathology. In: Brown RJ, Meininger V, Swash M, eds. *Amyotrophic Laeral Sclerosis*. London: Martin Dunitz, 2000; 83–112.
4. Patrikios JS. *Contribution À l'Étude Des Formes Cliniques Et De l'Anatomie Pathologique De La Sclérose Latérale Amyotrophique*. Paris University, 1918.
5. Bonduelle M. Amyotrophic lateral sclerosis. In: Vinken PJ, Bruyn GW, eds. *Handbook of Clinical Neurology*. 22. Amsterdam: North-Holland, 1975; 281–338.
6. Cappellari A, Ciammola A, Silani V. The pseudopolyneuritic form of amyotrophic lateral sclerosis (Patrikios' disease). *Electromyogr Clin Neurophysiol* 2008; **48**: 75–81.
7. Guidetti D, Bondavalli M, Sabadini R *et al.* Epidemiological survey of amyotrophic lateral sclerosis in the province of Reggio Emilia, Italy: influence of environmental exposure to lead. *Neuroepidemiology* 1996; **15**: 301–312.
8. Mortara P, Bardelli D, Leone M, Schiffer D. Prognosis and clinical varieties of ALS disease. *Ital J Neurol Sci* 1981; **2**: 237–242.
9. Salemi G, Fierro B, Arcara A, Cassata M, Castiglione MG, Savettieri G. Amyotrophic lateral sclerosis in Palermo, Italy: an epidemiological study. *Ital J Neurol Sci* 1989; **10**: 505–509.
10. Wijesekera LC, Mathers S, Talman P *et al.* Natural history and clinical features of the flail arm and flail leg ALS variants. *Neurology* 2009; **72**: 1087–1094.
11. Nishigaki S, Ando K, Nagata Y, Hirose K. Pseudopolyneuritic form of amyotrophic lateral sclerosis. *Rinsho Shinkeigaku* 1973; **13**: 377–384.
12. Terao S, Sobue G, Hashizume Y, Mitsuma T, Takahashi A. Disease-specific patterns of neuronal loss in the spinal ventral horn in amyotrophic lateral sclerosis, multiple system atrophy and X-linked recessive bulbospinal neuronopathy, with special reference to the loss of small neurons in the intermediate zone. *J Neurol* 1994; **241**: 196–203.
13. Tsuchiya K, Shintani S, Kikuchi M *et al.* Sporadic amyotrophic lateral sclerosis of long duration mimicking spinal progressive muscular atrophy: a clinicopathological study. *J Neurol Sci* 1999; **162**: 174–178.
14. Hasegawa M, Arai T, Nonaka T *et al.* Phosphorylated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Ann Neurol* 2008; **64**: 60–70.
15. Neumann M, Rademakers R, Roeber S, Baker M, Kretzschmar HA, Mackenzie IR. A new subtype of frontotemporal lobar degeneration with FUS pathology. *Brain* 2009; **132**: 2922–2931.
16. Hayashi S, Sakurai A, Amari M, Okamoto K. Pathological study of the diffuse myelin pallor in the anterolateral columns of the spinal cord in amyotrophic lateral sclerosis. *J Neurol Sci* 2001; **188**: 3–7.
17. Nishihira Y, Tan CF, Hoshi Y *et al.* Sporadic amyotrophic lateral sclerosis of long duration is associated with relatively mild TDP-43 pathology. *Acta Neuropathol* 2009; **117**: 45–53.
18. Brownell B, Oppenheimer DR, Hughes JT. The central nervous system in motor neurone disease. *J Neurol Neurosurg Psychiatry* 1970; **33**: 338–357.
19. Ince PG, Evans J, Knopp M *et al.* Corticospinal tract degeneration in the progressive muscular atrophy variant of ALS. *Neurology* 2003; **60**: 1252–1258.
20. Sasaki S, Iwata M. Immunocytochemical and ultrastructural study of the motor cortex in patients with lower motor neuron disease. *Neurosci Lett* 2000; **281**: 45–48.
21. Tan CF, Eguchi H, Tagawa A *et al.* TDP-43 immunoreactivity in neuronal inclusions in familial amyotrophic lateral sclerosis with or without SOD1 gene mutation. *Acta Neuropathol* 2007; **113**: 535–542.
22. Mackenzie IR, Bigio EH, Ince PG *et al.* Pathological TDP-43 distinguishes sporadic amyotrophic lateral sclerosis from amyotrophic lateral sclerosis with SOD1 mutations. *Ann Neurol* 2007; **61**: 427–434.
23. Aoki M, Ogasawara M, Matsubara Y *et al.* Familial amyotrophic lateral sclerosis (ALS) in Japan associated with H46R mutation in Cu/Zn superoxide dismutase gene: a possible new subtype of familial ALS. *J Neurol Sci* 1994; **126**: 77–83.
24. Ohi T, Saita K, Takechi S *et al.* Clinical features and neuropathological findings of familial amyotrophic lateral sclerosis with a His46Arg mutation in Cu/Zn superoxide dismutase. *J Neurol Sci* 2002; **197**: 73–78.

25. Ohi T, Nabeshima K, Kato S, Yazawa S, Takechi S. Familial amyotrophic lateral sclerosis with His46Arg mutation in Cu/Zn superoxide dismutase presenting characteristic clinical features and Lewy body-like hyaline inclusions. *J Neurol Sci* 2004; **225**: 19–25.

26. Arisato T, Okubo R, Arata H *et al.* Clinical and pathological studies of familial amyotrophic lateral sclerosis (FALS) with SOD1 H46R mutation in large Japanese families. *Acta Neuropathol* 2003; **106**: 561–568.

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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

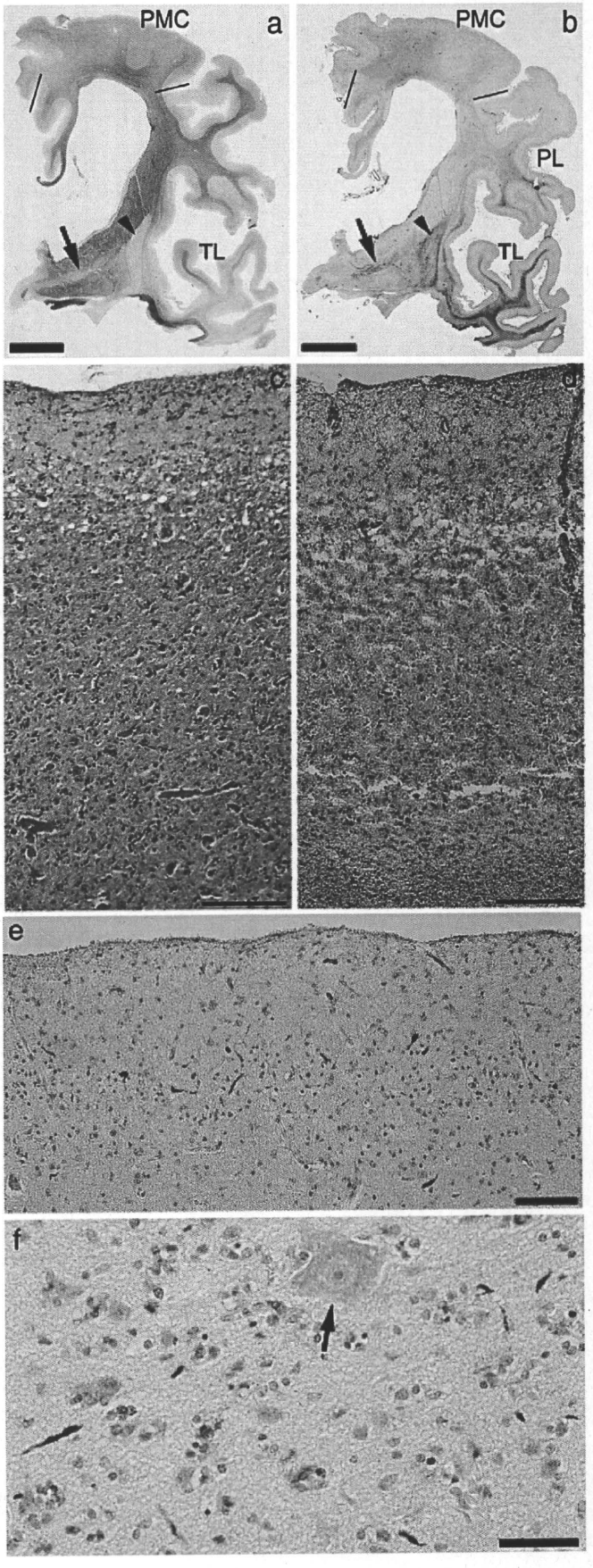

Fig. 1. (case 10) a and b are serial sections. a The left hemispheric section at the plane of the globus pallidus is shown. The area of cerebral cortex between the lines is the primary motor cortex (PMC) where Betz cells were identified. Cerebral atrophy was accentuated in the temporal lobe (TL). Enlargement 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.

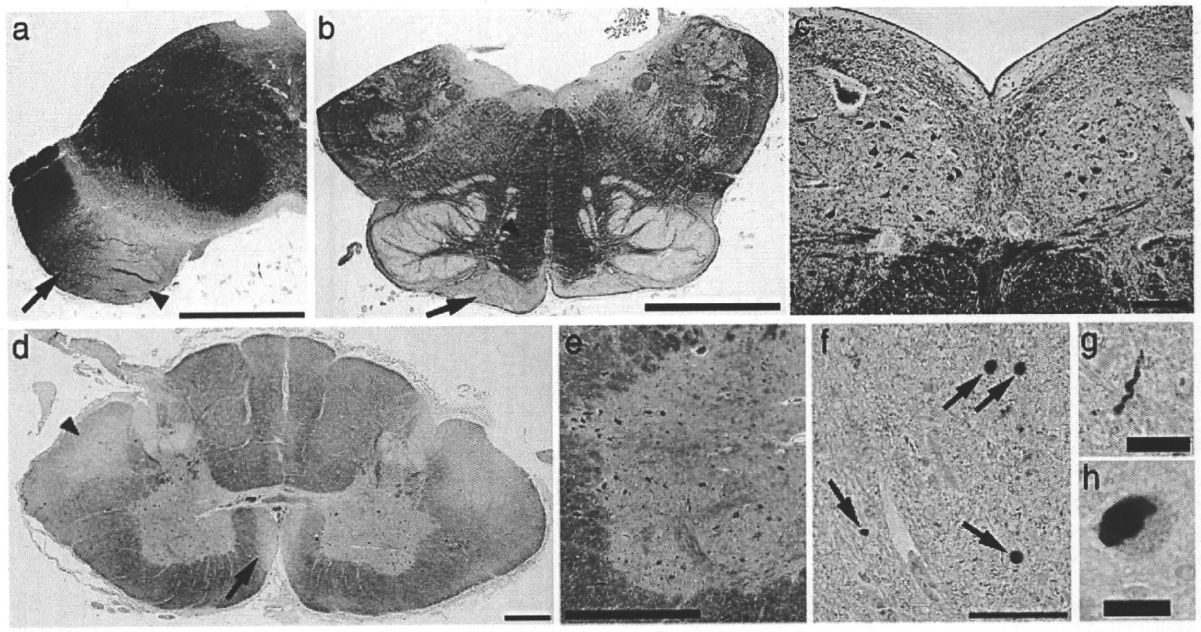

Fig. 2. (case 10) a Myelin pallor was demonstrated in the middle third of the cerebral peduncle corresponding to the corticospinal tract (CST) (arrow), although more marked degeneration was observed in the medial third of the cerebral peduncle corresponding to the frontopontine tract (arrowhead). b Atrophy 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.

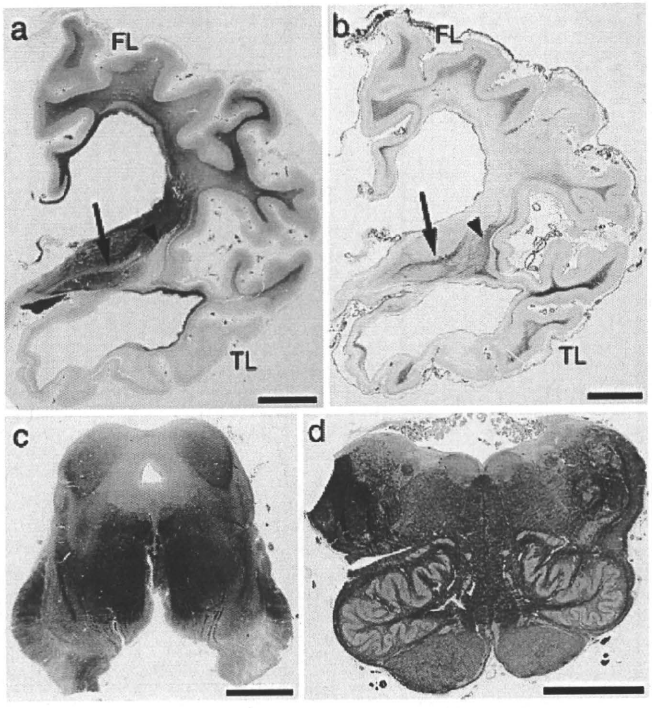

Fig. 3. a and b are serial sections of case 8. a The left hemispheric section at the plane of the posterior limb of the internal capsule showed the atrophy of the frontal lobe (FL), temporal lobe (TL) and putamen (arrowhead). The lateral ventricle was markedly enlarged. Myelin pallor was demonstrated 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

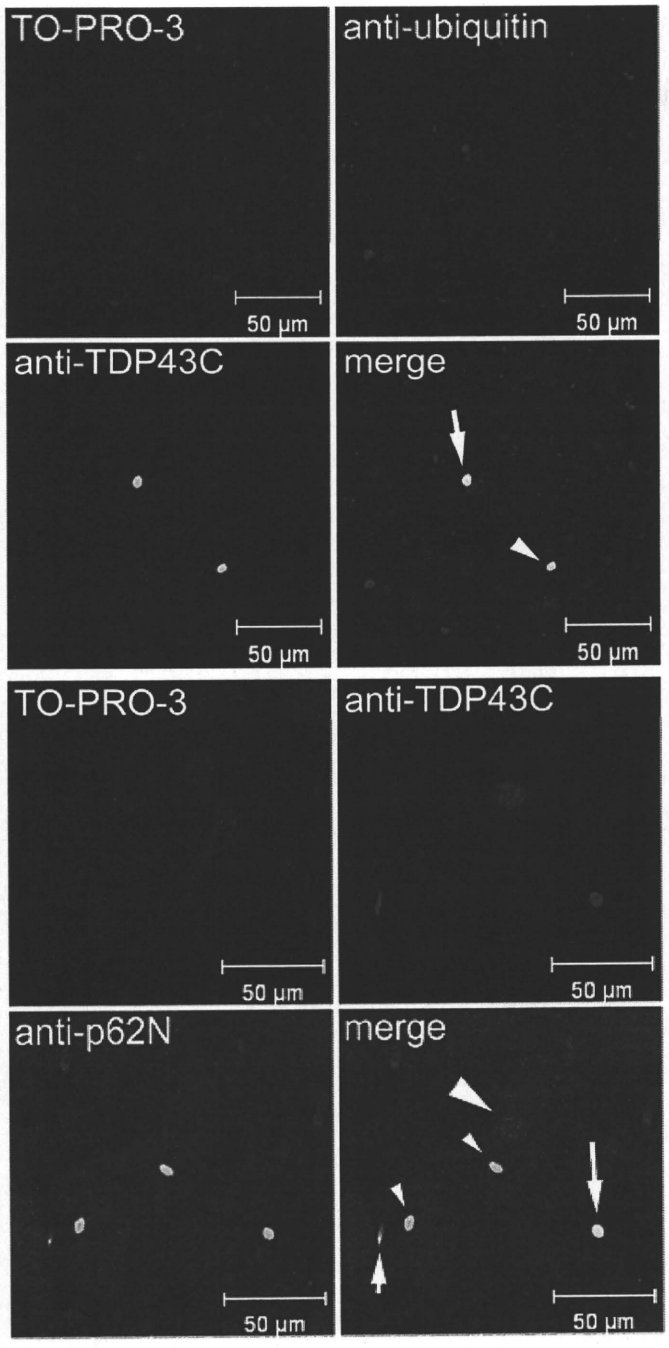

Fig. 4. The cervical cord anterior horn of case 10 was investigated. Nuclei were stained with TO-PRO-3 (Invitrogen, Tokyo, Japan), producing a blue color. Confocal immunofluorescence of ubiquitin and TDP-43C [405–414] showed colocalization in an NCI (arrow) and a round structure in the neuropil (arrowhead).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

Acknowledgement

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References

[1] Josephs KA, Stroh A, Dugger B, Dickson DW. Evaluation of subcortical pathology and clinical correlations in FTLD-U subtypes. Acta Neuropathol 2009;118:349–58.
[2] Snowden J, Neary D, Mann D. Frontotemporal lobar degeneration: clinical and pathological relationships. Acta Neuropathol 2007;114:31–8.
[3] Cairns NJ, Bigio EH, Mackenzie IR, Neumann M, Lee VM, Hatanpaa KJ, et al. Neuropathologic diagnostic and nosologic criteria for frontotemporal lobar degeneration: consensus of the Consortium for Frontotemporal Lobar Degeneration. Acta Neuropathol 2007;114:5–22.
[4] Igaz LM, Kwong LK, Xu Y, Truax AC, Uryu K, Neumann M, et al. Enrichment of C-terminal fragments in TAR DNA-binding protein-43 cytoplasmic inclusions in brain but not in spinal cord of frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Am J Pathol 2008;173:182–94.
[5] Hu WT, Seelaar H, Josephs KA, Knopman DS, Boeve BF, Sorenson EJ, et al. Survival profiles of patients with frontotemporal dementia and motor neuron disease. Arch Neurol 2009;66:1359–64.
[6] Toyoshima Y, Tan CF, Kozakai T, Tanaka M, Takahashi H. Is motor neuron disease-inclusion dementia a forme fruste of amyotrophic lateral sclerosis with dementia? An autopsy case further supporting the disease concept. Neuropathology 2005;25:214–9.
[7] Tan CF, Toyoshima Y, Kakita A, Takahashi H. Neuropathological similarities and differences between frontotemporal lobar degeneration with ubiquitin inclusions and amyotrophic lateral sclerosis with dementia (in Japanese). Brain Nerve 2009;61:1319–27.
[8] Mitsuyama Y, Inoue T. Clinical entity of frontotemporal dementia with motor neuron disease. Neuropathology 2009;29:649–54.
[9] Cairns NJ, Neumann M, Bigio EH, Holm IE, Troost D, Hatanpaa KJ, et al. TDP-43 in familial and sporadic frontotemporal lobar degeneration with ubiquitin inclusions. Am J Pathol 2007;171:227–40.
[10] Hasegawa M, Arai T, Nonaka T, Kametani F, Yoshida M, Hashizume Y, et al. Phosphorylated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Ann Neurol 2008;64:60–70.
[11] Mackenzie IR, Baborie A, Pickering-Brown S, Du Plessis D, Jaros E, Perry RH, et al. Heterogeneity of ubiquitin pathology in frontotemporal lobar degeneration: classification and relation to clinical phenotype. Acta Neuropathol 2006;112:539–49.
[12] Geser F, Martinez-Lage M, Robinson J, Uryu K, Neumann M, Brandmeir NJ, et al. Clinical and pathological continuum of multisystem TDP-43 proteinopathies. Arch Neurol 2009;66:180–9.
[13] Pikkarainen M, Hartikainen P, Alafuzoff I. Neuropathologic features of frontotemporal lobar degeneration with ubiquitin-positive inclusions visualized with ubiquitin-binding protein p62 immunohistochemistry. J Neuropathol Exp Neurol 2008;67:280–98.
[14] Grossman M, Wood EM, Moore P, Neumann M, Kwong L, Forman MS, et al. TDP-43 pathologic lesions and clinical phenotype in frontotemporal lobar degeneration with ubiquitin-positive inclusions. Arch Neurol 2007;64:1449–54.
[15] Standring S. Gray's Anatomy: The Anatomical Basis of Clinical Practice, Thirty-Ninth edition. United Kingdom: Churchill Livingstone; 2004. p. 397.
[16] Josephs KA, Dickson DW. Frontotemporal lobar degeneration with upper motor neuron disease/primary lateral sclerosis. Neurology 2007;69:1800–1.
[17] Yokota O, Tsuchiya K, Arai T, Yagishita S, Matsubara O, Mochizuki A, et al. Clinicopathological characterization of Pick's disease versus frontotemporal lobar degeneration with ubiquitin/TDP-43-positive inclusions. Acta Neuropathol 2009;117:429–44.
[18] Ikeda K, Akiyama H, Arai T, Ueno H, Tsuchiya K, Kosaka K. Morphometrical reappraisal of motor neuron system of Pick's disease and amyotrophic lateral sclerosis with dementia. Acta Neuropathol 2002;104:21–8.
[19] Ikeda K, Tsuchiya K, Akiyama H, Arai T, Matsushita M, Kosaka K. Reappraisal of Pick's disease. Where should cases of lober atrophy without Pick bodies be placed? (in Japanese). Shinkei Kenkyu no Shinpo 2001;25:329–41.
[20] Kosaka K, Ikeda K, Kobayashi K, Hamamoto J, Matsushita M. On pyramidal tract lesions in Pick's disease (in Japanese). Seishin Igaku 1985;27:1171–8.
[21] Tsuchiya K, Ikeda K, Mimura M, Takahashi M, Miyazaki H, Anno M, et al. Constant involvement of the Betz cells and pyramidal tract in amyotrophic lateral sclerosis with dementia: a clinicopathological study of eight autopsy cases. Acta Neuropathol 2002;104:249–59.
[22] Uchihara T, Sato T, Suzuki H, Ikeda K, Akiyama H, Takatori T. Bunina body in frontal lobe dementia without clinical manifestations of motor neuron disease. Acta Neuropathol 2001;101:281–4.
[23] Mackenzie IR, Neumann M, Bigio EH, Cairns NJ, Alafuzoff I, Kril J, et al. Nomenclature for neuropathologic subtypes of frontotemporal lobar degeneration: consensus recommendations. Acta Neuropathol 2009;117:15–8.
[24] Ikeda M, Ishikawa T, Tanabe H. Epidemiology of frontotemporal lobar degeneration. Dement Geriatr Cogn Disord 2004;17:265–8.
[25] Ikeda K. Neuropathological discrepancy between Japanese Pick's disease without Pick bodies and frontal lobe degeneration type of frontotemporal dementia proposed by Lund and Manchester Group. Neuropathology 2000;20:76–82.
[26] McKeith IG, Dickson DW, Lowe J, Emre M, O'Brien JT, Feldman H, et al. Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. Neurology 2005;65:1863–72.
[27] Josephs KA, Parisi JE, Knopman DS, Boeve BF, Petersen RC, Dickson DW. Clinically undetected motor neuron disease in pathologically proven frontotemporal lobar degeneration with motor neuron disease. Arch Neurol 2006;63:506–12.
[28] Mochizuki A, Komatsuzaki Y, Iwamoto H, Shoji S. Frontotemporal dementia with ubiquitinated neuronal inclusions presenting with primary lateral sclerosis and parkinsonism: clinicopathological report of an autopsy case. Acta Neuropathol 2004;107:377–80.
[29] Tsuchiya K, Murayama S, Mitani K, Oda T, Arima K, Mimura M, et al. Constant and severe involvement of Betz cells in corticobasal degeneration is not consistent with pyramidal signs: a clinicopathological study of ten autopsy cases. Acta Neuropathol 2005;109:353–66.
[30] Tsuchiya K, Piao YS, Oda T, Mochizuki A, Arima K, Hasegawa K, et al. Pathological heterogeneity of the precentral gyrus in Pick's disease: a study of 16 autopsy cases. Acta Neuropathol 2006;112:29–42.
[31] Yaguchi M, Okamoto K, Nakazato Y. Frontotemporal dementia with cerebral intraneuronal ubiquitin-positive inclusions but lacking lower motor neuron involvement. Acta Neuropathol 2003;105:81–5.
[32] Josephs KA, Katsuse O, Beccano-Kelly DA, Lin WL, Uitti RJ, Fujino Y, et al. Atypical progressive supranuclear palsy with corticospinal tract degeneration. J Neuropathol Exp Neurol 2006;65:396–405.
[33] Ince PG. Neuropathology. In: Brown RJ, Meininger V, Swash M, editors. Amyotrophic lateral sclerosis; 2000. p. 83–112. London.
[34] Kato S. Amyotrophic lateral sclerosis models and human neuropathology: similarities and differences. Acta Neuropathol 2008;115:97–114.

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Short communication

Frontotemporal lobar degeneration with motor neuron disease showing severe and circumscribed atrophy of anterior temporal lobes

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ABSTRACT

Frontotemporal lobar degeneration (FTLD) is characterized by a variety of behavioral and psychiatric symptoms based on the dysfunction of frontal and/or temporal lobes. A 63-year-old Japanese man without a family history of neurological diseases developed progressive symptoms of frontotemporal dementia, followed by motor neuron disease (MND). Brain magnetic resonance images demonstrated severe atrophy in the anterior temporal lobes from early clinical stage. The symptoms got rapidly worsened and the patient died of respiratory failure 1 year 8 months after the disease onset. A postmortem study revealed severe and circumscribed atrophy in the anterior temporal lobes, and histological examination disclosed marked neuronal loss with many neuronal cytoplasmic inclusions which were immunoreactive for ubiquitin antibodies and phosphorylated TAR DNA-binding protein of 43 kDa (TDP-43) antibodies in hippocampal dentate granule cells and amygdalae, as well as a few neuronal cytoplasmic inclusions without dystrophic neurites in the temporal neocortex. This case report showed typical features of FTLD–MND in clinical course and TDP-43 pathology with unusual severity and distribution of cerebral atrophy, suggesting a unique manifestation of FTLD–MND.

1. Introduction

Frontotemporal lobar degeneration (FTLD) is a common clinical entity of dementia characterized by behavioral and psychiatric symptoms resulting from dysfunction of frontal and/or temporal lobes [1]. Pathologically, FTLD comprises two major disease entities based on the chief component of neuronal and/or glial inclusions: one is tauopathy, which has recently been classified as FTLD-tau, such as Pick body disease, corticobasal degeneration, progressive supranuclear palsy, and argyrophilic grain dementia, and the other entity is TAR DNA-binding protein of 43 kDa (TDP-43) proteinopathy [2], or FTLD-TDP as the latest nomenclature, which is characterized by ubiquitin- and TDP-43-positive, tau-negative neuronal inclusions [3].

FTLD patients mainly present sporadic occurrence, though gene mutations including microtubule-associated protein tau (*MAPT*) [4], progranulin (*PRGN*) [5,6] or valosin-containing protein (*VCP*) [7] can be identified in familial cases. Some sporadic FTLD patients also have motor neuron disease (MND), especially amyotrophic lateral sclerosis (ALS) [1]. This type of FTLD is recognized as FTLD–MND, which demonstrates FTLD-TDP pathology [2]. Recent studies have revealed that the concept of FTLD–MND overlaps with that of ALS with dementia which has an emphasis on ALS as the main pathology [8], and have suggested that FTLD and ALS have the same pathophysiology associated with abnormal TDP-43 protein metabolism.

Here we report an autopsy case of FTLD–MND with unusual pathological feature, and discuss the pathophysiology in consideration of a wide variety of FTLD manifestations.

2. Clinical summary

The patient was a Japanese man with no family history of neurological diseases. He was in good health until the age of 63, when he felt difficulty in speaking. At the same time his family became aware of a change in his personality: he had been a gentle and quiet person with a career as a chief executive director of a high school, but he became irritable and short-tempered with his family and friends. On the first examination we found that the patient developed flaccid dysarthria, not aphasia, and that cognitive disturbances including disorientation and memory impairment were not evident. There was no atrophy, weakness, or fasciculation in the muscles of the patient's

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limbs, trunk, or tongue. Tendon reflexes were normal and pathological reflexes were absent.

Brain magnetic resonance images at this time demonstrated severe atrophy in the anterior portion of temporal lobes, predominantly on the right side (Fig. 1). In frontal, parietal, and occipital lobes, atrophy was unremarkable. Cerebellum and brainstem were intact. Blood flow was decreased bilaterally in the anterior temporal lobes on ^{123}I-IMP single photon emission computed tomography. At this moment, we assessed the patient's condition to be unusual for Alzheimer's disease in both symptoms and radiological findings.

About one year after the first symptom emerged, the patient began to complain difficulty in swallowing and muscle weakness in the limbs. Physical examinations disclosed muscle atrophy and weakness with fasciculation in the limbs and tongue. Tendon reflexes were diminished in all limbs, and dysarthria was worsened with a reduction in loudness. He got more irritable, often impatient at our interviews or examinations, and remarkably careless about the advice from his family and friends even in his daily life. Neuropsychological investigations proved no evidence of disorientation, episodic memory impairment, semantic memory impairment, spatial agnosia, apraxia, oral tendency, grasping reflex, compulsive manipulation of tools, hallucination, delusion, apathy, depression, obstinate imitation, or stereotypic behavior. The patient answered correctly both in naming test as for objects and fingers and in facial recognition test as for famous persons. The patient was not aware of the personality change himself.

Muscle atrophy and weakness rapidly worsened, and the patient became bedridden about 1 year 5 months after the first symptom appeared. Tube feeding was started at the same period because of severe dysphagia. The patient died of respiratory failure without artificial ventilation about 1 year 8 months after the disease onset, at the age of 65.

3. Neuropathological findings

The brain weighed 1240 g after fixation. Macroscopic examination showed severe atrophy in the anterior portion of the temporal lobes, predominantly on the right side (Fig. 2A–D). Atrophy was also prominent in the bilateral parahippocampal gyri and amygdalae. Depigmentation was clearly observed in the substantia nigra. Cerebellum, pons, and medulla oblongata had no remarkable changes.

Histological examination revealed marked neuronal loss with gliosis bilaterally in the first temporal gyri (Fig. 2E, F), cingulate gyri, insular gyri, parahippocampal gyri, subiculum, basolateral group of amygdalae, and substantia nigra. Some section of the first temporal gyri had degeneration in all layers, and free melanins were abundantly present in the substantia nigra (Fig. 3A). Neuronal loss was only slightly observed in the frontal and occipital lobes, and the Betz cells in the primary motor cortex were mostly preserved. We did not find neuronal loss in the caudate, putamen, thalamus, oculomotor nuclei, trochlear nuclei, abducens nuclei, locus ceruleus, pontine nuclei, dentate nuclei, inferior olive, dorsal vagal nuclei, or Purkinje cells in the cerebellum. Degeneration of pyramidal tracts was detected only in the lumber spinal cord. Neuronal loss with presence of Bunina bodies and spheroids was noticeable in the hypoglossal nuclei (Fig. 3B–D) and anterior horn cells of the lumbar spinal cord. There were no Pick bodies or ballooned neurons in any section of the brain. Posterior columns, Onuf's nuclei and dorsal nuclei of Clarke in the spinal cord were intact.

Immunohistochemistry using antibodies to ubiquitin (Z0458, rabbit, polyclonal, 1:5000, Dako, Glostrup, Denmark) showed neuronal cytoplasmic inclusions abundantly in hippocampal dentate granule cells (Fig. 3E) and amygdalae (Fig. 3F). Some of these inclusions were also immunoreactive for phosphorylated TDP-43 antibodies (pS409/410, rabbit, polyclonal, 1:1000 [9]) (Fig. 3G). We identified no dystrophic neurites, but a few neuronal cytoplasmic inclusions, which were positive for both ubiquitin and phosphorylated TDP-43, in the temporal neocortex. Though there were a few neurofibrillary changes, consistent with Braak stage I, in the hippocampus and parahippocampal gyri, we did not find a neuronal or glial inclusion that was positively stained by phosphorylated tau antibody (AT8, mouse, monoclonal, 1:3000, Innogenetics, Ghent, Belgium), such as tuft-shaped astrocyte, coiled body, or argyrophilic thread. In the substantia nigra, there were no structures stained by phosphorylated alpha-synuclein antibodies (1175, rabbit, polyclonal, 1:1000 [10]).

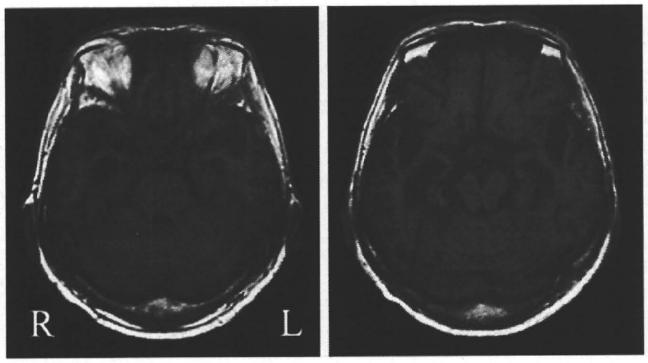

Fig. 1. T1-weighted magnetic resonance images show severe atrophy in the anterior temporal lobes at the early clinical stage.

4. Discussion

FTLD is clinically classified into three subtypes: frontotemporal dementia (FTD), progressive non-fluent aphasia (PNFA), and semantic dementia (SD) [1]. Our patient demonstrated FTD symptoms characterized by personality change and disordered social conduct, such as disinhibition, irritability, and lack of insight. The patient had no impairment in semantic memory, such as word meaning aphasia or prosopagnosia, suggesting that his symptom was not appropriate as SD. PNFA was also considered to be inappropriate because his language disturbance was not aphasia but progressive dysarthria. FTLD–MND patients mostly have FTD symptoms [1], whereas PNFA is extremely rare [11] and SD is not reported. Therefore, our patient presenting with FTD symptoms was typical as a FTLD–MND patient.

Pathological findings revealed ubiquitin- and TDP-43-positive neuronal inclusions in the hippocampal dentate granule cells, amygdalae, and temporal neocortex without tau-positive constructs such as Pick bodies or glial fibrillary tangles, except for a few neurofibrillary changes. These findings were consistent with FTLD-TDP pathology. The morphology of ubiquitin- and TDP-43-positive neuronal inclusions in FTLD-TDP cases contains three major patterns: neuronal cytoplasmic inclusions (NCIs), neuronal intranuclear inclusions (NIIs), and dystrophic neurites (DNs). Accordingly, FTLD-TDP is classified into four types based on these morphologic patterns and regional distribution of inclusions in brain pathology [2]. Our case disclosed frequent NCIs with no NIIs or DNs in the regions mentioned above, which involved typical characteristics of type 2. Most FTLD–MND cases show type 2 pathology with a few cases showing type 3 pathology, which is characterized by numerous DNs and NCIs, predominantly in the superficial cortical layers, and is known as the pathology observed in FTLD cases with *PRGN* mutations [2]. To our knowledge, there is no report of a FTLD–MND case showing type 1 pathology, which is characterized by an abundance of long DNs, predominantly in the superficial cortical laminae, or type 4 pathology, which is distinguished by numerous NIIs and infrequent DNs and/or NCIs in the neocortical areas, and is described in FTLD cases with *VCP* mutations [12]. Consequently, our case was typical as a FTLD–MND case in the aspect of TDP-43 pathology, besides the clinical course.

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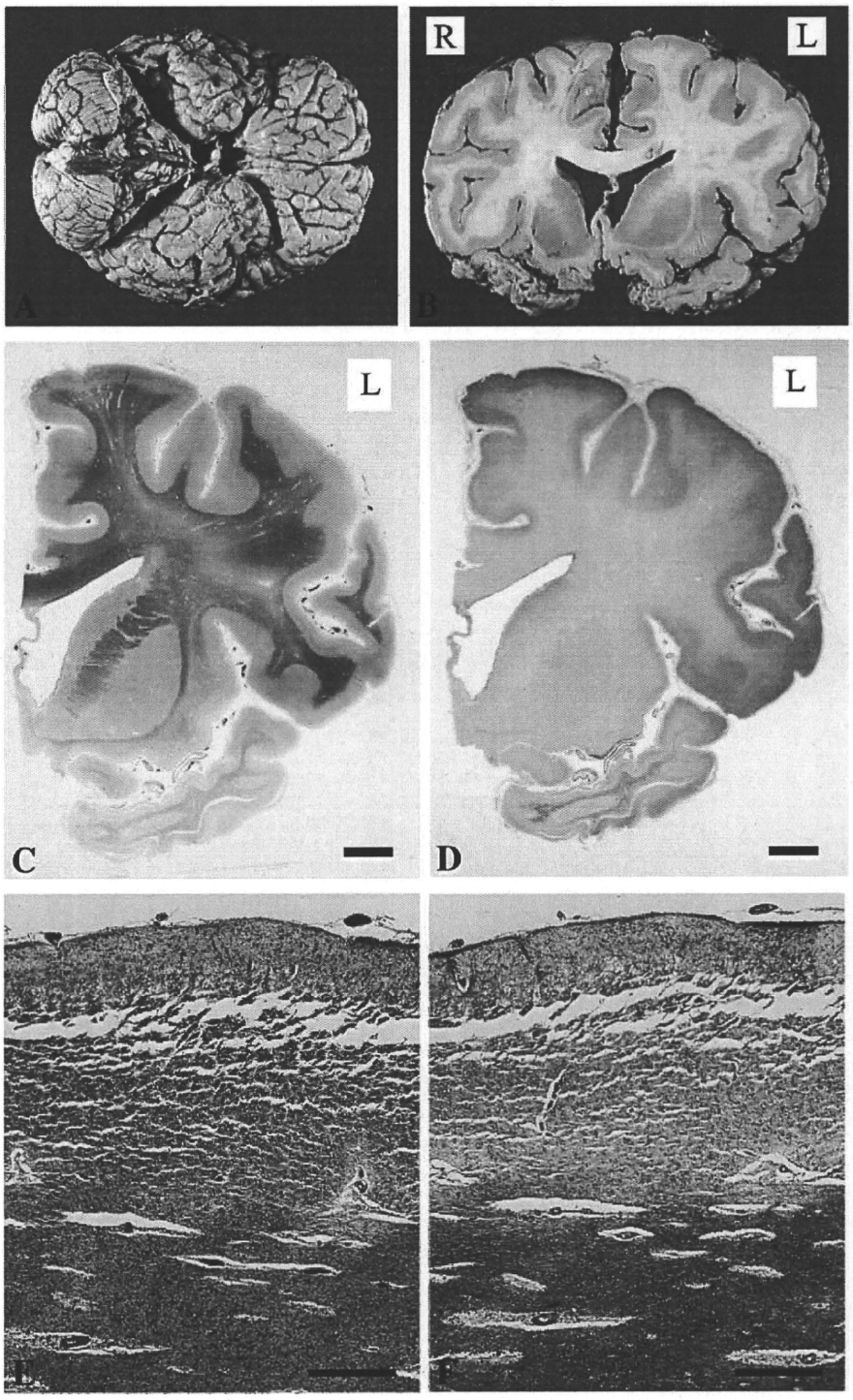

Fig. 2. A: Macroscopic examination of the brain reveals atrophy in the anterior temporal lobes. B: Coronal section through the anterior horn of lateral ventricle shows severe atrophy of the temporal lobes, predominantly on the right side. C, D: Coronal section through the anterior portion of the left temporal lobe shows prominent atrophy with loss of myelin and fibrillary gliosis. E, F: The first temporal cortex on the left side shows marked neuronal loss (E) and fibrillary gliosis (F). C: Klüver–Barrera staining. D, F: Holzer staining. E: Hematoxylin and eosin staining. Bars, C, D: 10 mm, E, F: 500 μm.

Our case had an outstanding feature of severe and circumscribed atrophy in the anterior temporal lobes from the early clinical stage. In typical FTLD–MND cases, the severity of frontal and/or temporal lobe atrophy is limited to be relatively mild [13], possibly due to the shortness of disease duration (mean 2.3 years) compared to FTLD cases without MND (mean 8.6 years) [14]. Moreover, circumscribed lobar atrophy, which is classically known as a frequent observation in Pick body disease, is quite rare in FTLD–MND cases [13]. Therefore, our case was unusual for FTLD–MND in the manifestation of cerebral atrophy.

Generally, circumscribed atrophy of anterior temporal lobes is recognized as a major feature of FTLD cases with SD symptoms [15,16]. Especially, prosopagnosia is a frequent SD symptom in the cases exhibiting severe atrophy on the right side, whereas word meaning aphasia is common when atrophy is predominant on the left side [17,18]. In our patient presenting predominant atrophy on the

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