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INTERNAL MEDICINE

□ CASE REPORT □

Familial ALS with G298S Mutation in *TARDBP*: A Comparison of CSF Tau Protein Levels with those in Sporadic ALS

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Abstract

We report a 52-year-old Japanese man showing both upper and lower motor neuron signs with familial amyotrophic lateral sclerosis (ALS). Analysis of the TAR DNA-binding protein of 43 kDa (TDP-43) gene (*TARDBP*) revealed a glycine-to-serine substitution at position 298 (G298S). Cerebrospinal fluid (CSF) level of total tau protein (CSF-tau) of our patient was found to be highly elevated compared with those of sporadic ALS cases and controls. The elevated CSF-tau level might be related to the damage of neurons exhibiting a large number of TDP-43 inclusions in familial ALS with this mutation.

Key words: familial amyotrophic lateral sclerosis, TAR DNA binding protein gene, tau, cerebrospinal fluid

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Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by the degeneration of motor neurons in the brain and spinal cord. In ALS, lower motor neurons exhibit ubiquitinated neuronal inclusions (UNIs), which are also detected in the brain in cases of frontotemporal lobar degeneration (FTLD) (1). TAR DNA-binding protein of 43 kDa (TDP-43) has been identified as the major component of UNIs, and both sporadic ALS (SALS) and FTLD are considered to involve a common pathological mechanism (1). This group of neurological diseases that are associated with TDP-43 accumulation are referred to as TDP-43 proteinopathies (1).

Familial ALS (FALS) is observed in 5 to 10% of all ALS cases, and exhibits an autosomal dominant inheritance (2). The most common cause of FALS was reported to be mutations in the Cu/Zn superoxide dismutase gene (*SOD-1*); however, *SOD-1* mutations have been found in about 20% of FALS cases (2). Recently, FALS cases with mutations in the TAR DNA-binding protein gene (*TARDBP*) which encodes TDP-43 have been reported (3-10). We report a Japanese FALS patient with a mutation in *TARDBP*; we examined the levels of cerebrospinal fluid (CSF)-amyloid β protein 1-42 (CSF-$A\beta_{42}$), CSF-total tau protein (CSF-tau), and CSF-phosphorylated tau protein (CSF-ptau) in our patient compared with SALS patients and controls.

Case Report

A 52-year-old Japanese man first noticed difficulty moving his right thumb, and fasciculation in all limbs and trunk developed within one month. Over the following month, the

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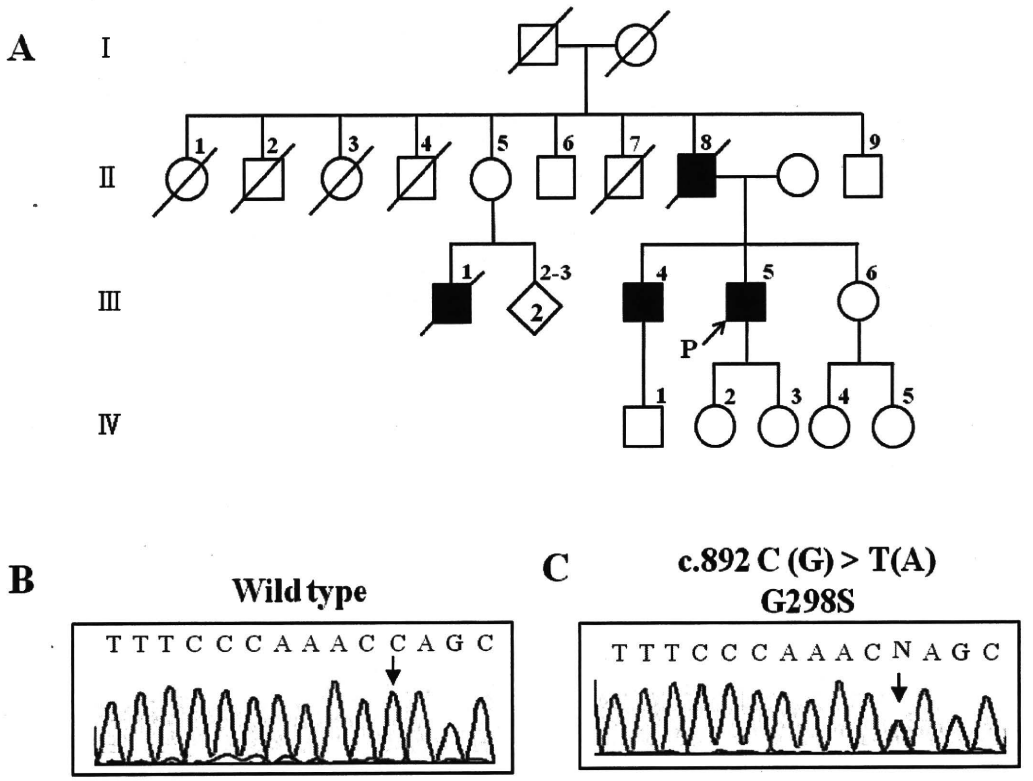

Figure 1. (A) The pedigree of our patient's family. The proband (our patient III-5) is marked with an arrow. Roman numerals indicate generations, and Arabic numerals at the upper right of the symbols indicate individuals. The symbols are as follows: squares, males; circles, females; diamond shape, individuals for whom gender was not disclosed. Multiple siblings are indicated with a number inside the symbols. Shading of the symbols indicates a diagnosis of ALS (our patient's father II-8, our patient's brother III-4 and our patient's cousin III-1). The *TARDBP* was only analyzed in our patient (III-5). (B and C) Sequencing chromatogram of a part of *TARDBP* in a sample from a control (B) and our patient (C). The chromatogram shows the heterozygous sequence trace of C (G) to T (A) at complementary DNA position c.892 for genotyping by reverse primer. The nucleotide position of the substitution is indicated by an arrow.

weakness of his right hand progressed, and cramp of the lower limbs developed. Neurological examination performed three months after the onset revealed mild weakness of the right abductor pollicis brevis, opponens pollicis, and extensor hallucis brevis; hyperreflexia in all extremities with positive right Babinski and Chaddock signs; and fasciculation in all extremities and the back. The patient's sensory perception and coordination were unremarkable. A laboratory test showed a mild elevation of serum creatinine phosphate kinase level (450 IU/L; normal, 45-163 IU/L). CSF examination showed a normal cell count and protein level. Magnetic resonance images of brain and spinal cord disclosed no remarkable findings. Electromyography revealed active denervation and renervation discharges in muscles of all limbs and the tongue. Wechsler Adult Intelligence Scale-Third Edition (WAIS-III) indicated a full scale Intelligence Quotient (IQ) 109, verbal IQ 94, and performance IQ 106. The results of a four-factor model were as follows: verbal comprehension, 93; perceptual organization, 108; working memory, 102; and processing speed, 102. We diagnosed definite ALS according to the revised El Escorial criteria (11).

The patient was treated with 100 mg/day of riluzole; however, during the year following disease onset, weakness rapidly progressed, and the patient's gait became disturbed. The patient died due to respiratory failure 15 months after disease onset.

The patient's father (II-8), brother (III-4), and cousin (III-1) had suffered from ALS (Fig. 1A). The father (II-8) developed weakness of the right hand at age 45, which spread to his right leg, and lead to a bed-ridden state for a half-year period. The father died two years after disease onset. The brother (III-4) developed weakness of the left leg at age 54, and, 8 months after the diagnosis, he required wheelchair and noninvasive positive pressure ventilation. We could not obtain detailed information of the cousin (III-1) who had been diagnosed with ALS. There was little information of the cousin's mother (II-5) who had not developed ALS when the patient was admitted to our hospital. The family history suggests a diagnosis of FALS with autosomal dominant inheritance.

Genomic DNA was purified from whole blood. All exons and exon-intron boundaries of *SOD-1* (12) and *TARDBP* were analyzed with PCR and direct sequencing. Sequencing of *TARDBP* was performed for our patient and for 96

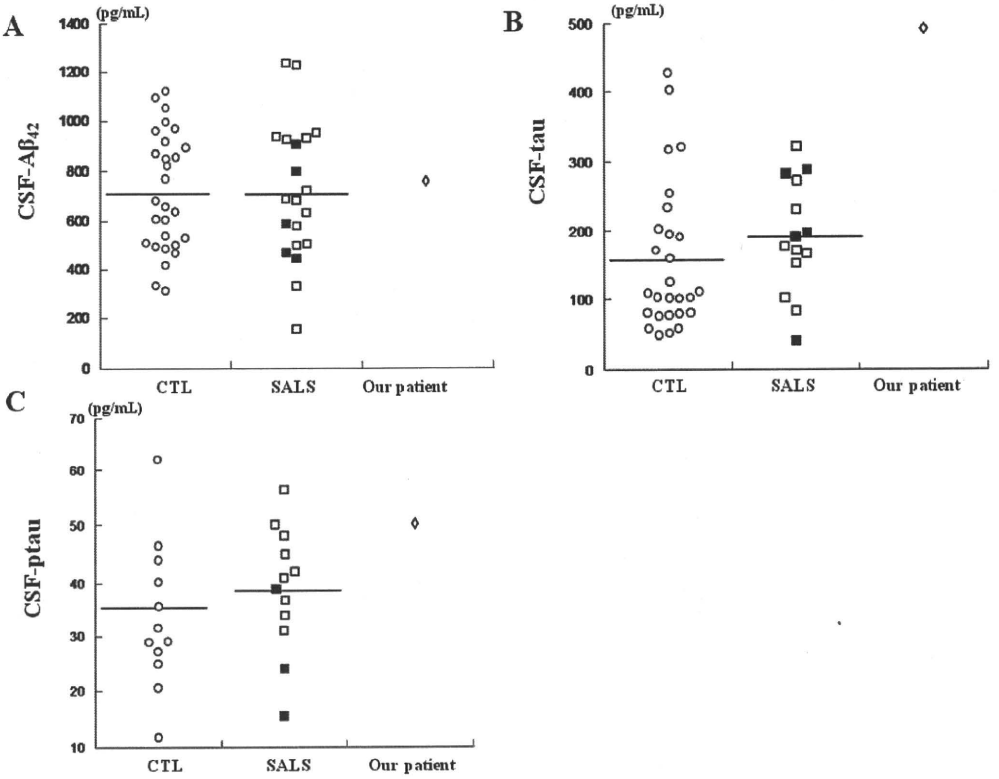

Figure 2. (A, B, and C) Comparison of cerebrospinal fluid (CSF)-amyloid β protein 1-42 (CSF-$A\beta_{42}$) (A), total tau protein (CSF-tau) (B), and phosphorylated tau protein (CSF-ptau) (C) among controls (CTL), sporadic amyotrophic lateral sclerosis (SALS) cases, and our patient. In addition to our patient, 20 patients with SALS [age, 60.8±11.0 years; clinical duration (from disease onset to admission), 23.3±21.7 months] and 27 age-matched CTL without disorders of the central nervous system (age, 53.8±17.4 years) were included in the study. Bars show the mean value. Five SALS patients with relatively rapid progression (duration of clinical course <10 months) are indicated by solid squares (see text).

healthy Japanese subjects. The genomic structure of *TARDBP* (RefGene NM_007375) was confirmed from the University of California, Santa Cruz database (http://www.genome.ucsc.edu/) and the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/>). All participants provided informed consent. The present study was approved by the ethics committees of all participating institutions.

The levels of CSF-$A\beta_{42}$, CSF-tau, and CSF-ptau in which tau protein is phosphorylated at Thr181, were measured in the following groups as previously reported (13): our patient; 20 patients with SALS; and 27 age-matched controls without disorders of the central nervous system.

Sequencing of *SOD-1* revealed no mutations. Sequencing of the coding regions of *TARDBP* revealed a heterozygous G-to-A transition at complementary DNA position 892 (c.892 G>A), which leads to the substitution of glycine by serine at position 298 (G298S) in a highly conserved region within exon 6 (Figs. 1B, 1C). We also searched for this mutation in 96 healthy controls, and none demonstrated this mutation.

When CSF-$A\beta_{42}$, CSF-tau, and CSF-ptau levels were compared between SALS cases and controls, the levels of CSF-$A\beta_{42}$ [n=20, 712.0±280.9 (mean ± standard deviation) (range, 159-1,243) pg/mL], CSF-tau [n=14, 190.6±82.7 (39-321) pg/mL], and CSF-ptau [n=12, 38.6±11.3 (15.6-56.0) pg/mL] in the SALS cases were not significantly different from those in the controls [CSF-$A\beta_{42}$, n=27, 711.7±241.5 (337-1,126) pg/mL; CSF-tau, n=27, 156.3±106.9 (53-428) pg/mL; and CSF-ptau, n=13, 35.3±14.2 (12.0-62.1) pg/mL] (Fig. 2A-C). In our patient, the concentrations of CSF-$A\beta_{42}$ (764 pg/mL) and CSF-ptau (50 pg/mL) were similar to those of the SALS cases and the controls (Figs. 2A, 2C); however, the concentration of CSF-tau (491 pg/mL) was elevated by comparison (+3SD compared with the mean value of those in the controls) (Fig. 2B). In five patients with SALS who showed relatively rapid progression (duration from onset to CSF examination <10 months), the concentration of CSF-tau was 199.6±100.8 (range, 39-282) pg/mL, which was not significantly different from those in the other nine SALS patients (Fig. 2B).

Discussion

More than 10 mutations in *TARDBP* have been identified in FALS and SALS cases (3-10). We have described a Japanese FALS patient without dementia with a G298S missense mutation of *TARDBP*. To date, this mutation has only been

reported in a Chinese FALS family (3). The family with this mutation was reported to exhibit disease onset at between 41-60 years of age with subsequent rapid progression (mean duration, 2 years) (4). The present patient and affected family members demonstrated similar ages at disease onset, and similar clinical durations (<2 years) to the reported cases (3). The aunt (II-5) [i.e. the mother of affected cousin (III-1)] was a healthy carrier, and this incomplete penetrance was compatible with the previous report (3).

CSF-tau is considered to reflect neuronal damage, but it is controversial whether CSF-tau concentration is elevated in SALS cases compared with controls (14-17). In our study, the CSF-tau levels did not differ between SALS and controls; however, the CSF-tau level of our patient was found to be elevated compared with those of SALS cases and controls. The CSF-tau levels were not associated with severity of disease progression in the SALS patients as reported in a previously report (17), and the duration of the clinical course from onset to CSF examination in our patient (three months) was similar to those in the five SALS patients with relatively rapid progression (5.6±0.9 months; range, 4-6 months). Thus, the elevated CSF-tau level cannot be simply attributed to rapid disease progression of FALS with the G298S mutation of *TARDBP*. Although there was no quantitative pathological analysis of TDP-43 positive inclusions and pre-inclusions, the reported FALS patients with the G298S mutation of *TARDBP* were reported to have more TDP-43 positive pre-inclusions in many areas of the central nervous system compared with SALS patients (3). We speculate that tau protein may be easily released from damaged motor neurons with TDP-43 positive inclusions to extracellular space. In addition, the CSF-ptau levels were not changed in this FALS patient as well as in SALS patients, suggesting the absence of hyperphosphorylated tau pathology in ALS. The CSF-$A\beta_{42}$ level has previously been reported to be significantly decreased in ALS cases compared with controls (16); in our study, however, CSF-$A\beta_{42}$ levels in our patient and in SALS patients were not significantly different from those of controls. The previous reports on FALS or SALS with *TARDBP* mutations including the G298S mutation presented no data about CSF-tau, CSF-$A\beta_{42}$, or CSF-ptau (3).

Finally, the G298S mutation of *TARDBP* has only been identified in FALS patients with an Asian ethnic background (3). It remains to be determined whether this mutation is also present in populations with other ethnic backgrounds.

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NEUROPATHOLOGY

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Case Report

Morel's laminar sclerosis showing apraxia of speech: Distribution of cortical lesions in an autopsy case

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A 57-year old man with chronic alcoholism presented with apraxia of speech and disturbance of consciousness. He had a history of gastrectomy and had been drinking alcohol. The symptoms improved with administration of thiamine, but he later developed diarrhea and delirium, and died approximately 40 days after the onset. Autopsy findings were consistent with Wernicke's encephalopathy and pellagra encephalopathy. Furthermore, laminar cortical necrosis with vacuoles and astrocytosis was found in the second and third layers of the bilateral frontal cortices, suggesting Morel's laminar sclerosis. The lesions were mainly located in the bilateral primary motor cortices. Involvement of the lower part of the left primary motor cortex may be associated with apraxia of speech in our case.

Key words: apraxia of speech, Morel's laminar sclerosis, pellagra encephalopathy, primary motor cortex, Wernicke's encephalopathy.

INTRODUCTION

A brief summary of the clinicopathological features of this case was previously reported in this journal as "Neuropathology Education" by Tsuchiya, one of the authors of this paper.[1] This time, we have added the description of the clinical course, pathological findings, distribution of the cortical lesions, and review of the literature regarding Morel's laminar sclerosis, and discuss matters related to clinical practice.

The term Morel's laminar sclerosis was derived from the publication by Morel in 1939 describing unusual pathological changes in four alcoholics, taking the form of spongiform change and gliosis of the cerebral cortex, mostly confined to the third layer.[2] Although some reports described patients with Morel's laminar sclerosis,[3–6] the information regarding clinical and pathological findings has been limited.

Apraxia of speech (AOS) is caused by disturbance of motor programming of articulation, and is classically distinguished from Broca's aphasia by the preservation of the ability to write language.[7] Here, we report a patient presenting with AOS, and having histological findings indicating Morel's laminar sclerosis, as well as Wernicke's encephalopathy and pellagra encephalopathy.

CASE REPORT

The patient was a 57-year-old Japanese man, who had been living alone. After the publication of the previous paper,[1] we found that the patient had been drinking 80–100 g of alcohol per day (The duration was unclear.). Past history included diabetes mellitus, foot necrosis, angina pectoris, and gastric cancer. Because of these diseases, coronary artery bypass graft (at age 51), digital amputation (at age 55), and total gastrectomy (at age 55) were performed. Five

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days before admission, his brother had contact with the patient by telephone, and the patient had been normal. On the day of admission, an acquaintance visited him and found him lying near his bed. He opened his eyes, but could not speak. On admission, body temperature was 36.0°C, blood pressure 136/115 mmHg, and heart rate 101 beats/min. General examination showed severe emaciation and surgical scars on the chest and abdomen. The palpebral conjunctiva was anemic. The second and third digits of the right foot had been amputated. On neurological examination, consciousness disturbance (easy arousal after normal calling), and hyporeflexia in the four extremities were demonstrated. He could not speak, but comprehension of spoken language was normal. Tongue protrusion was impossible. External ocular movement was normal. There was no motor paralysis in the face or limbs. Ataxia of the upper limbs was not apparent, while that of the lower limbs and trunk could not be examined. Blood test showed a white blood cell (WBC) of 7100/mm^3 (normal range: 4000–9000), red blood cell (RBC) 237 × 10^4/mm^3 (normal range: 430–570 × 10^4), platelet count (Plt) 14.9 × 10^4/mm^3 (normal range: 15–35 × 10^4), total protein 6.3 g/dL (normal range: 6.5–8.0), urea nitrogen 41.6 mg/dL (normal range 8–20), creatinine 0.7 mg/dL (normal range: 0.6–1.2), aspartate aminotransferase (AST) 58 IU/L (normal range: < 35), alanine aminotransferase (ALT) 45 IU/L (normal range: < 40), blood sugar 180 mg/dL, and C-reactive protein 0.9 mg/dL. Brain CT demonstrated mild atrophy at the bilateral frontal and temporal lobes. Although blood thiamine was not measured, the diagnosis of Wernicke's encephalopathy was suspected based on the history of gastrectomy and drinking alcohol. Hydration with administration of thiamine was initiated, but niacin was not given. Consciousness disturbance gradually improved, but anterograde and retrograde amnesia, and time disorientation became evident. At that time, we found that his written language was normal, although the disturbance in speech output was not completely resolved. Diarrhea was also seen after the initiation of oral intake, but he was transferred to another hospital 30 days after admission. Thereafter, he developed delirium and convulsion, and was transferred back to our hospital 6 days later. He died on the same day. Artificial ventilation was not administered throughout the course. Autopsy was limited to the brain.

The brain weighed 1130 g after fixation. Macroscopically, bilateral mamillary bodies and inferior colliculi appeared gray. There were no abnormalities in the thalamus or cerebral cortex (Fig. 1). Atherosclerotic changes of the middle cerebral arteries and basilar artery were mild. Brain tissue samples were fixed post mortem with 10% formalin and embedded in paraffin. Ten-μm-thick hemispheric sections were prepared at the plane of the left frontal lobe (the most anterior section), left amygdala, bilateral mamillary bodies, left pulvinar nucleus, and right occipital lobe. The sections of upper and lower portions of the midbrain, upper and middle portions of the pons, upper, middle, and lower portions of the medulla oblongata, and bilateral cerebellum were also prepared. These sections were stained with HE, KB, Bodian, and Gallyas method, and anti-glial fibrillary acidic protein (GFAP) (rabbit, polyclonal, Dako, Glostrup, Denmark, 1:1000). Reticulin silver stain was also performed to visualize the capillaries.

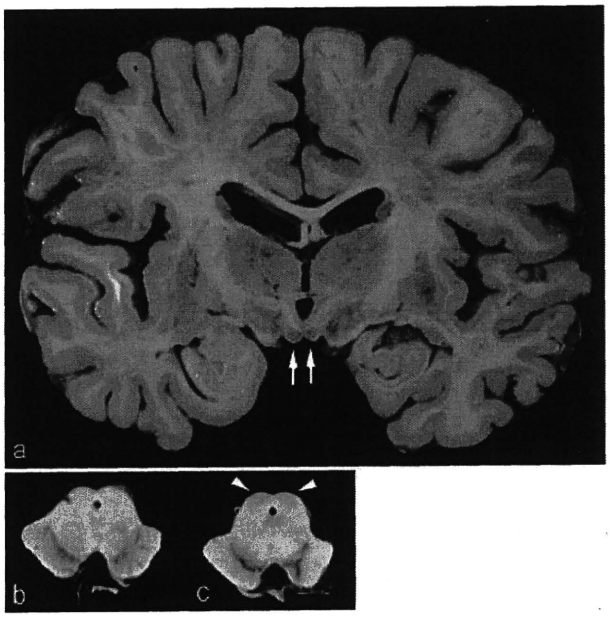

Fig. 1 (a,c) Grayish discoloration in the bilateral mamillary bodies (a, arrows) and inferior colliculi (c, arrowheads). (b) At the level of the upper midbrain. There are no macroscopic abnormalities in the periaqueductal gray matter, thalamus or cerebral cortex.

Microscopic examination demonstrated necrotic foci with many vacuoles, accompanied by proliferation of macrophages and astrocytes, and capillary proliferation in the bilateral mamillary bodies and left inferior colliculi (Figs 2,3). Pinpoint hemorrhages were seen in the right mamillary body (Fig. 2c) and bilateral inferior colliculi. Neurons were relatively preserved in the lesion (Fig. 2d). Periaqueductal gray matter and oculomotor nucleus were not involved. In the thalamus, neuronal loss with astrocytosis was obvious in the bilateral mediodorsal nuclei, anterior nuclei (Fig. 3c), and left pulvinar nuclei. In the bilateral inferior olivary nucleus, patchy neuronal loss (Fig. 3d) with astrocytosis was evident. There were no abnormalities in the hypoglossal nucleus or cerebellum. These findings suggested the diagnosis of Wernicke's encephalopathy. In addition, central chromatolysis of the neurons without astrocytic or microglial changes was evident in the Betz

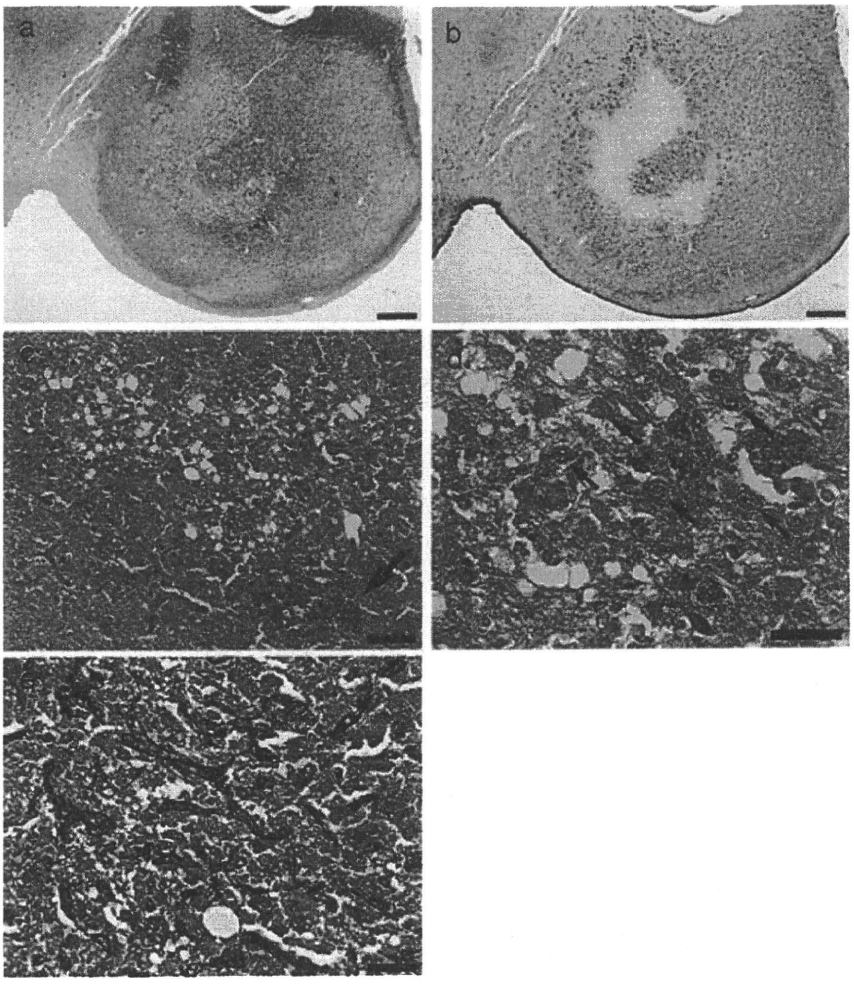

Fig. 2 (a) and (b) are serial sections. (a) Necrotic lesions in the right mamillary body. Bar = 500 μm. (b) Astrocytosis and central necrosis. Bar = 500 μm. (c) Many vacuoles and hemorrhages (arrow) in the right mamillary body. Bar = 100 μm. (d) Relative preservation of the neurons (arrows) in the right mamillary body. Bar = 50 μm. (e) Capillary proliferation in the right mamillary body. Bar = 50 μm. (a) KB stain, (b) GFAP stain, (c, d) HE stain, (e) Reticulin silver stain.

cells (Fig. 3e), pontine nucleus (Fig. 3f), left abducens nucleus, and bilateral cuneate nucleus, indicating pellagra encephalopathy. Furthermore, laminar cortical necrosis with vacuoles and astrocytosis was found in the second and third layers of the bilateral frontal cortices (Fig. 4). The vacuoles were partly seen in the first layer. The distribution of cortical lesions is shown in Figure 5. The lesions were mainly located in the bilateral primary motor cortices. On the left side, the lower part of the primary motor cortex was involved. The deep layers of the cortex were preserved. Neither thrombi nor atherosclerotic changes were observed in the vessels adjacent to the lesions. Alzheimer's type-II astrocytes were absent in the cerebral cortex and basal ganglia. There were no ischemic changes in the hippocampal pyramidal cells or cerebellar Purkinje cells. The Braak stage of neurofibrillary tangles was stage I. There were no Lewy bodies or Pick bodies. In other words, cortical changes cannot be explained by ischemia, hypoxia, hepatocerebral degeneration, or neurodegenerative diseases.

DISCUSSION

Clinically, our patient presented with AOS that improved after the administration of thiamine. He also developed diarrhea and delirium probably associated with pellagra. At autopsy, the diagnosis of Wernicke's encephalopathy and pellagra encephalopathy was confirmed. Furthermore, laminar cortical necrosis with vacuoles and astrocytosis was found in the second and third layers of the bilateral frontal cortices, suggesting Morel's laminar sclerosis.

Thiamine deficiency, which is considered a cause of Wernicke's encephalopathy, damages the mamillary bodies, medial thalamus, and periaqueductal gray matter. Other areas that are sometimes affected include the corpora quadrigemina, reticular formation of the midbrain, pontine tegmentum, superior vermis of the cerebellum,[8] and inferior olivary nucleus.[9] Observed lesions include loosening of the neuropil and vascular changes such as hemorrhage, capillary proliferation/dilatation, and endothelial swelling in the mamillary bodies and the subependymal structures

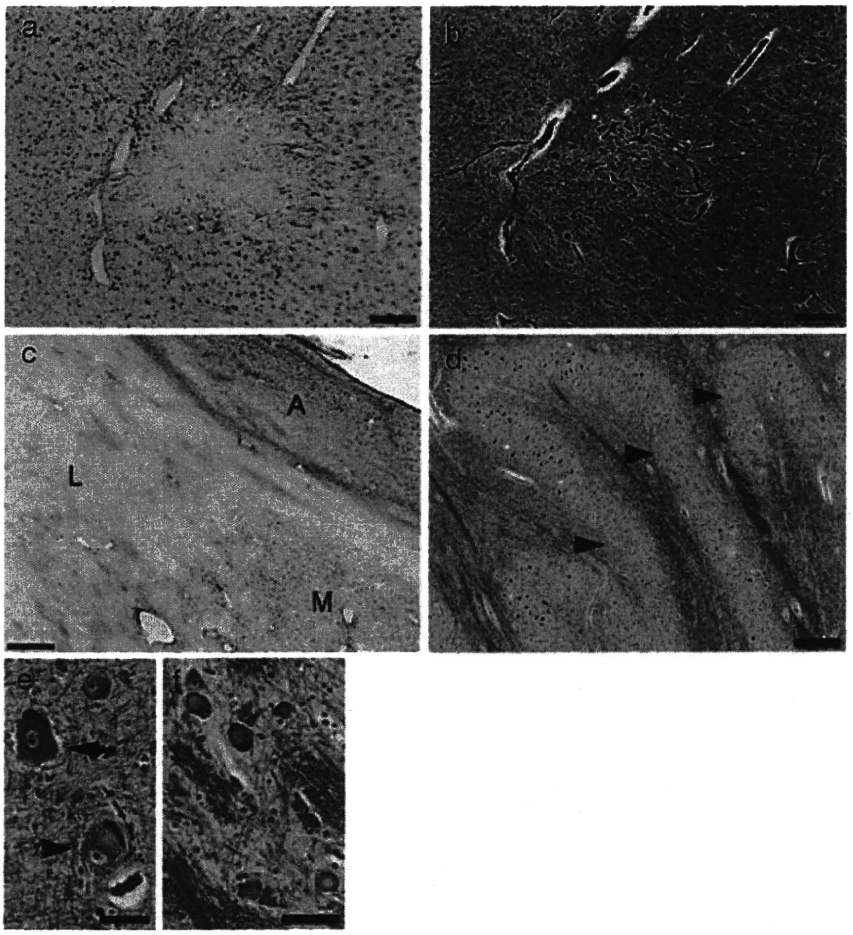

Fig. 3 (a) and (b) are serial sections. (a,b) Central necrosis with astrocytosis (a) and capillary proliferation (b) in the left inferior colliculi. Bar = 200 μm. (c) Astrocytosis in the mediodorsal nucleus (indicated with M) and anterior nucleus (indicated with A) of the right thalamus, with sparing of the lateral nucleus (indicated with L). Bar = 500 μm. (d) Patchy neuronal loss in the right inferior olivary nucleus (arrowhead). Bar = 200 μm. (e) Central chromatolysis of the Betz cell (arrowhead) and a normal Betz cell (arrow). Bar = 50 μm. (f) Central chromatolysis of the neurons in the pontine nucleus. Bar = 50 μm. (a, c) GFAP stain, (b) Reticulin silver stain, (d–f) KB stain.

along the aqueduct and fourth ventricle.[9,10] In the thalamus and inferior olivary nucleus, by contrast, neuronal loss is demonstrated with sparing of the neuropil and capillaries.[9] The distribution of lesions is associated with abundant thiamine-related glucose and oxygen metabolism.[11] Most surgical procedures that include gastrectomy, gastrojejunostomy, colectomy, gastric bypass surgery are risk factors for the development of Wernicke's encephalopathy.[8]

Various CNS disorders are seen in patients with Wernicke's encephalopathy. They include Marchiafava-Bignami disease,[6] pellagra encephalopathy,[12] and Morel's laminar sclerosis.[5] To date, pathomechanisms of Marchiafava-Bignami disease or Morel's laminar sclerosis have not been fully understood, although direct intoxication of alcohol or thiamine deficiency is considered to cause these diseases, while pellagra is thought to be caused by niacin deficiency.

Pellagra is classically characterized by a triad of dermatitis, diarrhea and dementia (or derilium), but dermatitis and diarrhea are often lacking.[13] Coexistence of Wernicke's encephalopathy or Marchiafava-Bignami disease makes the diagnosis of pellagra difficult. Based on the alcoholic background, thiamine therapy is started in most patients, but niacin is rarely started. Furthermore, antagonism between thiamine and niacin had also been suggested. The metabolic demands from a relative excess of thiamine may increase the requirements for pyridine coenzymes, nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). These require niacin as a cofactor. Serudaru *et al.* reported a case of a "late-onset" pellagra encephalopathy in an alcoholic patient who deteriorated after thiamine therapy.[13] This case and our case suggested that multiple vitamin therapy should be started when treating undiagnosed encephalopathies in patients with chronic alcoholism.

Pellagra encephalopathy presents with various symptoms such as dementia (or derilium), hypertonus, and myoclonus.[13] Serudaru *et al.* reported that hypertonus and myoclonus were associated with lesions of pontine nucleus and cerebellar dentate nucleus, respectively,[13] but correlation between the symptoms and brain lesions has not been fully established. Histopathologically, pellagra

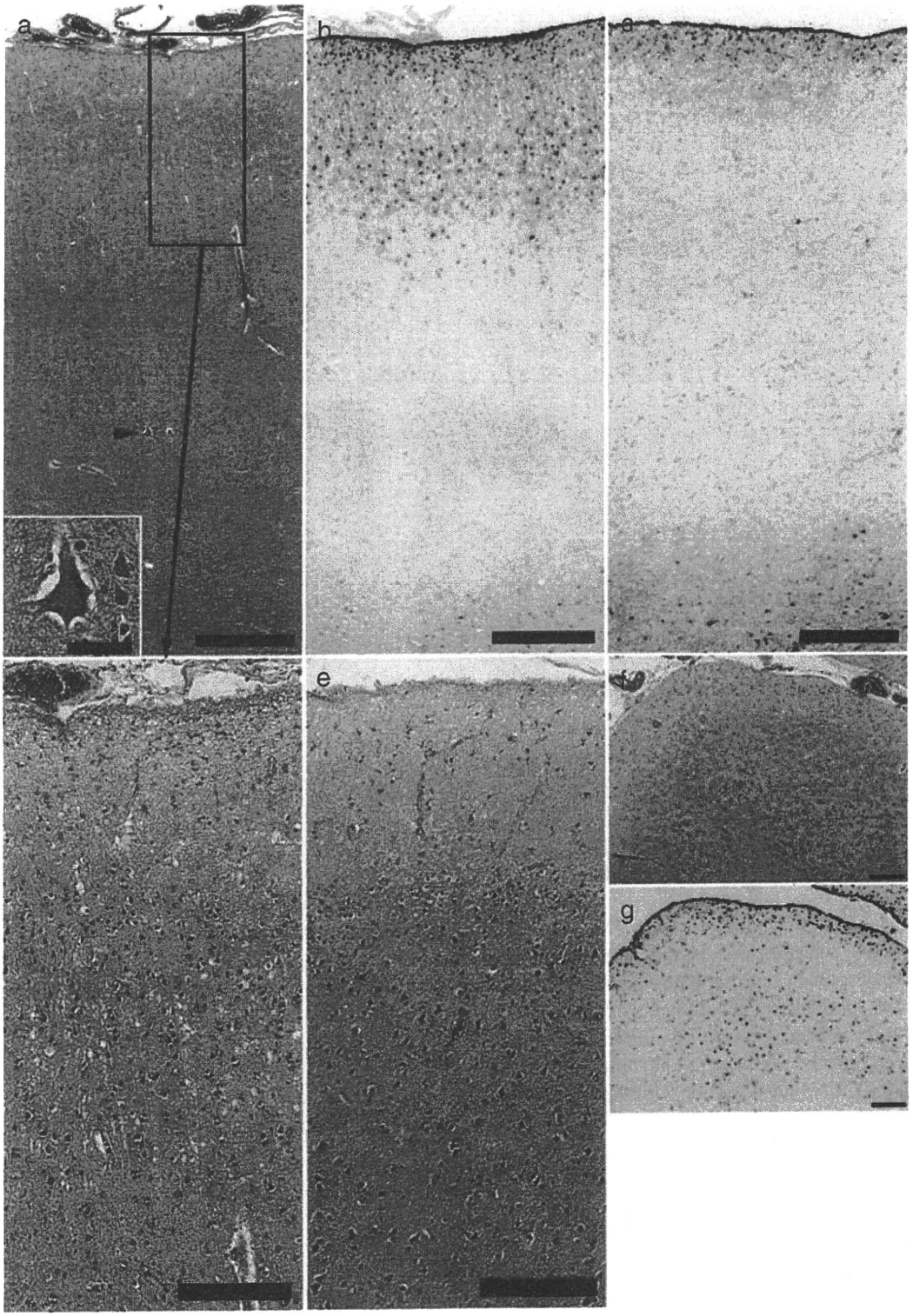

Fig. 4 (a) and (b) are serial sections. (a,b) Laminar cortical necrosis with vacuoles (a) and astrocytosis (b) in the second and third layers of the left primary motor cortex, with some vacuoles in the first layer (a). Bar = 500 μm. The arrowhead in (a) indicates a Betz cell located in the fifth layer. *Inset* shows this Betz cell. Bar = 50 μm. The area surrounded by the rectangle in (a) is shown in (d). (c) Normally, astrocytes are present only in the first layer of the cerebral cortex (the left postcentral gyrus of the patient). Bar = 500 μm. (d) Neuronal loss in the second and third layers. Bar = 200 μm. (e) There are no abnormalities in the second and third layers of the left postcentral gyrus. Bar = 200 μm. (f), (g) Another cortical lesion. Bar = 200 μm. (a), (d–f) HE stain, (b, c, g) GFAP stain.

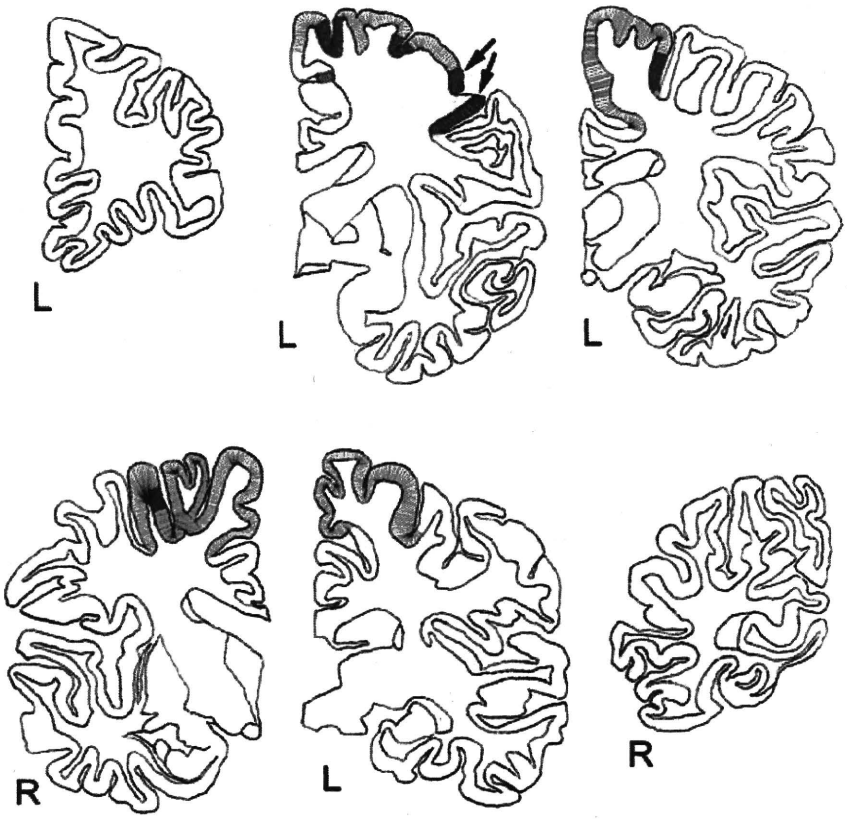

Fig. 5 The primary motor cortices where Betz cells were seen are depicted with lines. The distribution of laminar cortical necrosis with vacuoles and astrocytosis is shown in red. The lesions were mainly located in the bilateral primary motor cortices. On the left side, the lower part of the primary motor cortex was involved (arrows).

encephalopathy is characterized by central chromatolysis of the neurons in the pontine nucleus, where they are constant, in the cerebellar dentate nuclei, nuclei of cranial nerves, gracile and cuneate nuclei, and Betz cells.[14] The central chromatolysis in pellagra is thought to be not a retrograde change but a primary cytoplasmic change.[14]

Morel's laminar sclerosis was derived from the publication by Morel describing unusual pathological changes in four alcoholics, demonstrating spongiform change and astrocytosis seen extensively and symmetrically in the cerebral cortex, mostly confined to the third layer.[2] The review of Morel's laminar sclerosis published by Okeda *et al.* in 1976[5] described 21 cases of Morel's laminar sclerosis, in which Marchiafava-Bignami disease was seen in 17 cases (81%), and Wernicke's encephalopathy was seen in six cases (30%). They reported that Morel's laminar sclerosis was not seen in the occipital lobe. In their own case, laminar astrocytosis was seen in the third layer of the frontal, parietal, and temporal cortex. Spongiform changes were observed in the adjacent second layer. The lesions were severe in the middle and inferior frontal gyri.[5] Since 1976, three papers have reported patients with Morel's laminar sclerosis in the English language literature.[3,4,6] Naeije *et al.* reported that the lesions selectively involved the third and fourth layers of the cortex. Characteristic changes were neuronal loss, capillary proliferation with thickened endothelia, proliferation of microglial cells, and swollen astrocytes.[3] Okeda *et al.* reported that the cerebral cortex presented extensive laminar astrocytosis and/or laminar neuronal loss accompanied by capillary proliferation in the middle layers of the frontal and parietal cortices in case 2.[4] Sato *et al.* reported that the cerebral cortices showed mild neuronal loss and proliferation of astrocytes, particularly in the third layer.[6]

Recently, by application of MRI, cortical involvement in patients with Wernicke's encephalopathy or Marchiafava-Bignami disease has been increasingly reported.[11,15–22] Interestingly, the primary motor cortex was involved in most of these cases while the description regarding focal neurological signs was absent. In other words, the regions around the primary motor cortex seem to be sometimes mildly involved in these diseases. Although these reports did not include detailed histological findings, the cortical lesions may reflect Morel's laminar sclerosis[17] or hepatocerebral degeneration.[23]

To date, clinicopathological correlation in Morel's laminar sclerosis has rarely been reported, probably because consciousness disturbance due to concomitant Wernicke's encephalopathy or Marchiafava-Bignami disease masks the symptoms. In our case, spontaneous

speech was impossible on admission, but comprehension of spoken language was good. After the disappearance of consciousness disturbance, we found that the written language was normal, even though the disturbance in speech output was not completely resolved. Although neuropsychological tests were not performed in detail, we considered that his speech disturbance was AOS. The concept of AOS has been controversial and is sometimes called pure anarthrica[24] or aphemia.[7,25] AOS is caused by disturbance of motor programming of articulation, and is classically distinguished from Broca's aphasia by the preservation of ability to write language.[7] The differentiation of AOS from dysarthria is also sometimes difficult. In our case, tongue protrusion was impossible, but speech disturbance was significantly worse than expected. Although AOS has been reported to be caused by brain lesions involving various areas,[7,24–27] one of the responsible areas is the lower part of the left primary motor cortex.[25,27]

In conclusion, we report a patient with Morel's laminar sclerosis in whom AOS was seen. Involvement of the lower part of the left primary motor cortex may be associated with AOS in our case.

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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.

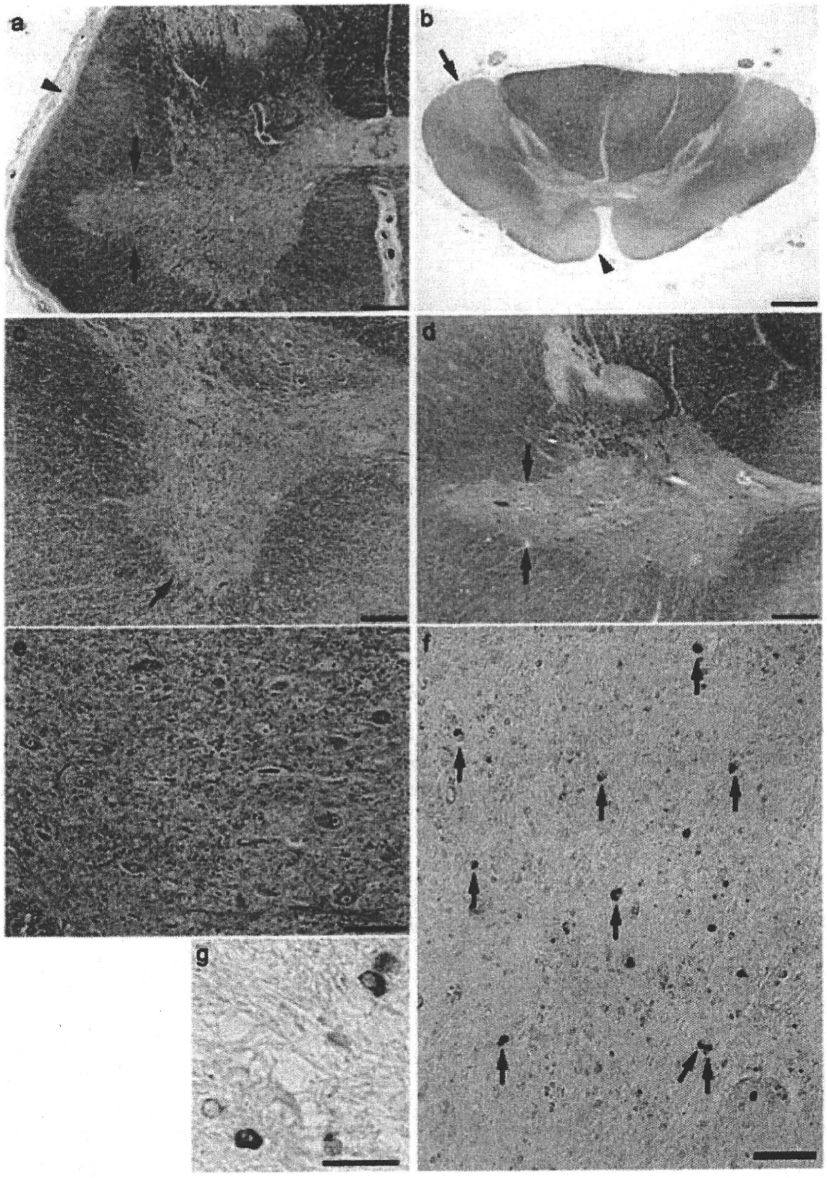

Fig. 1 Front and back diameter of the lateral part of the anterior horn was reduced in lumbar segment 5 (a, arrows). Motor neurons had almost disappeared (a). Lateral corticospinal tract (CST) showed myelin pallor and atrophy (a, arrowhead). In thoracic segment 10, myelin pallor was seen in the lateral (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

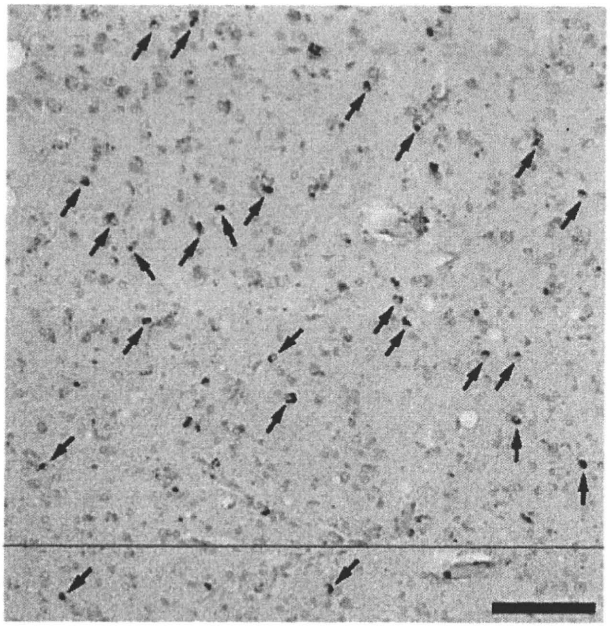

Fig. 2 A red line shows the boundary between the gray and white matter of the precentral gyrus. Immunohistochemistry using anti-phosphorylated TDP-43 (pS409/410) antibodies demonstrated that glial cytoplasmic inclusions (GCIs) (arrows) were frequently seen in the deep layer of the cortex, and less distributed 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

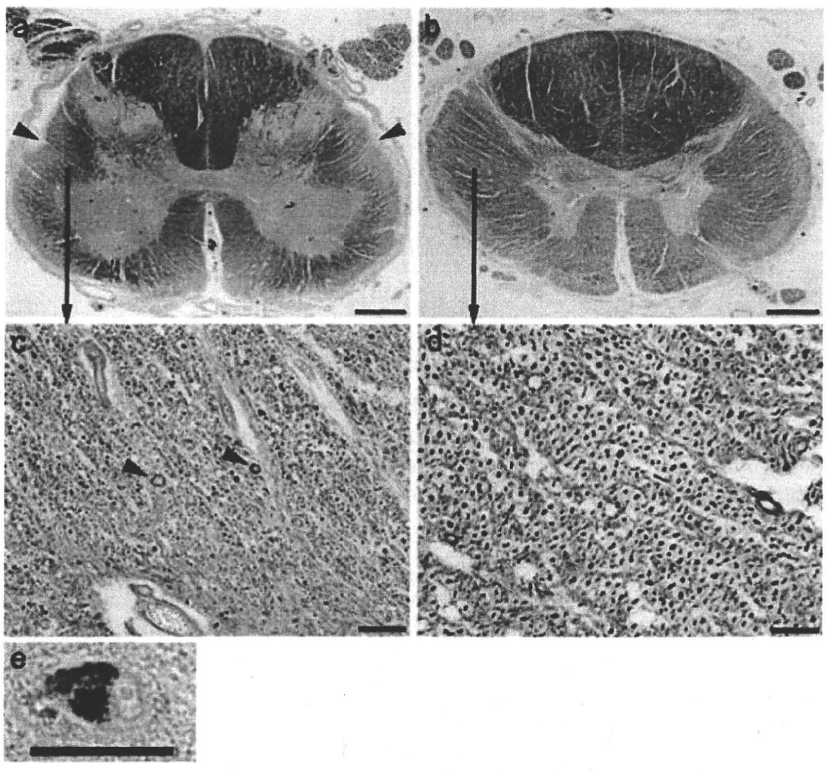

Fig. 3 Myelin pallor was demonstrated in the lateral corticospinal tract (CST) in lumbar segment 5 (a, arrowheads), but was not apparent in thoracic segment 10 (b). Axons were severely lost, and spheroids (c, arrowheads) were seen in the right lateral CST of lumbar segment 5 (c). In contrast, axons were 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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