BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

Differential motor neuron involvement in progressive muscular atrophy: a comparative study with amyotrophic lateral sclerosis

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2014-005213
Article Type:	Research
Date Submitted by the Author:	07-Mar-2014
Complete List of Authors:	Riku, Yuichi; Nagoya University Graduate School of Medicine, Department of Neurology Atsuta, Naoki; Nagoya University Graduate School of Medicine, Department of Neurology Yoshida, Mari; Aichi Medical University, Institute for Medical Science of Aging, Department of Neuropathology Tatsumi, Shinsui; Aichi Medical University, Institute for Medical Science of Aging, Department of Neuropathology Iwasaki, Yasushi; Aichi Medical University, Institute for Medical Science of Aging, Department of Neuropathology Mimuro, Maya; Aichi Medical University, Institute for Medical Science of Aging, Department of Neuropathology Watanabe, Hirohisa; Nagoya University Graduate School of Medicine, Department of Neurology Ito, Mizuki; Nagoya University Graduate School of Medicine, Department of Neurology Senda, Jo; Nagoya University Graduate School of Medicine, Department of Neurology Nakamura, Ryoichi; Nagoya University Graduate School of Medicine, Department of Neurology Koike, Haruki; Nagoya University Graduate School of Medicine, Department of Neurology Sobue, Gen; Nagoya University Graduate School of Medicine, Department of Neurology
Primary Subject Heading:	Neurology
Secondary Subject Heading:	Pathology
Keywords:	Neuropathology < NEUROLOGY, Motor neurone disease < NEUROLOGY, Adult neurology < NEUROLOGY

SCHOLARONE™
Manuscripts

Differential motor neuron involvement in progressive muscular atrophy: a comparative study with amyotrophic lateral sclerosis

Yuichi Riku[1]*, Naoki Atsuta[1]*, Mari Yoshida[2], Shinsui Tatsumi[2], Yasushi Iwasaki[2], Maya Mimuro[2], Hirohisa Watanabe[1], Mizuki Ito[1], Jo Senda[1], Ryoichi Nakamura[1], Haruki Koike[1], and Gen Sobue[1]

* These authors contributed equally to this work.

1. Department of Neurology, Nagoya University Graduate School of Medicine, Nagoya, Japan

2. Institute for Medical Science of Aging, Aichi Medical University, Aichi, Japan

Address for correspondence: Gen Sobue, MD, Department of Neurology, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan

E-mail: sobueg@med.nagoya-u.ac.jp; Fax: +81-52-744-2384; Phone: +81-52-744-2385

Word counts: Title 111 characters, Abstract 249 words, Manuscript 3376 words, Refs 25, Tables 1, and Figures 4.

Keywords: ALS, FUS, motor neuron disease, autopsy, PMA, TDP-43

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

ABSTRACT

Objective: Progressive muscular atrophy (PMA) is a clinical diagnosis characterized by progressive lower motor neuron (LMN) symptoms/signs with sporadic adult onset. Several studies have indicated that PMA often exhibits pathological features of amyotrophic lateral sclerosis (ALS). However, it is unclear whether PMA is simply a clinical phenotype of ALS in which upper motor neuron (UMN) signs are undetectable.

Methods: We compared clinicopathological profiles of clinically diagnosed PMA and ALS using 107 consecutive autopsied patients. For the clinical analysis, 14 and 103 patients were included in clinical PMA and ALS groups, respectively. For the neuropathological evaluation, 13 clinical PMA patients and 29 clinical ALS patients were included. Degeneration in the UMN and LMN systems, axonal density in the cortico-spinal tracts, and immunohistochemical profiles were evaluated.

Results: On the clinical evaluation, no significant difference between the prognosis of clinical PMA and ALS groups. On the pathological evaluation, 85% of clinical PMA patients showed degeneration in the UMN system. The large axon density in the cortico-spinal tracts of clinical PMA patients was higher on average than that of clinical ALS patients ($p = 0.001$). Immunohistochemically, 85% of clinical PMA patients displayed TDP-43-positive inclusions, while 15% displayed fused-in-sarcoma (FUS)-positive basophilic inclusion bodies. All of the clinical ALS patients displayed both UMN and LMN degeneration and TDP-43-positive inclusions.

For peer review only

Conclusions: PMA consists of three neuropathological patterns: UMN and LMN degeneration with TDP-43-pathology, consistent with ALS; LMN degeneration with TDP-43-pathology but sparing of the UMN system; and UMN and LMN degeneration with FUS-positive basophilic inclusion bodies.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

ARTICLE SUMMARY

Strengths and limitations of this study

Strengths:

#1. The characteristics of motor neuron involvement in ALS or PMA were comprehensively described.

#2. The severity of upper motor neuron involvement was semi-quantitatively evaluated and surrogated by axonal density of the pyramidal tract.

#3. The pathological results clearly indicated that the upper motor degenerations were significantly sparse in some PMA patients, compared to ALS.

Limitations:

#1. Inability to evaluate all the fields of the entire motor cortex and corticospinal tract.

#2. For quantification of axonal density in the corticospinal tract, we conducted immunohistochemical assay on paraffin embedded tissues using anti-neurofilament antibody. In this protocol, the tissues can shrink in some degrees, compared to epon embedded tissues.

For peer review only

INTRODUCTION

Motor neuron disease (MND) constitutes a group of heterogeneous neurodegenerative diseases that are associated with progressive upper (UMN) and / or lower motor neuron (LMN) degeneration. A portion of MND cases have genetic causes; however, the majority of MND cases are sporadic and of unknown etiology. Amyotrophic lateral sclerosis (ALS) constitutes the majority of MND cases. ALS is a clinicopathological disorder that clinically presents with progressive UMN and LMN symptoms / signs. Neuropathologically, both the UMN and LMN systems exhibit neuronal loss and gliosis, and Bunina bodies are detected in surviving neurons. Although various immunohistochemical profiles have been identified in ALS patients, 43-kDa TAR DNA-binding protein (TDP-43) is the major pathological protein in sporadic ALS.[1]

In contrast, MND that presents with LMN symptoms / signs alone occurs in several disorders, including the genetically mediated disorders spinal muscular atrophy (SMA), symmetrical axonal neuropathy, and spinal and bulbar muscular atrophy (SBMA).[2, 3] Additionally, a sporadic and adult-onset LMN disease has been referred to as progressive muscular atrophy (PMA).[3, 4] Although the revised El Escorial criteria, the standard diagnostic criteria for ALS, exclude patients who only present with LMN symptoms / signs, several studies have revealed that a subset of clinically diagnosed PMA patients exhibit the neuropathological hallmarks of ALS. Post-mortem histopathological studies have revealed cortico-spinal tract (CST) degeneration in more than half of the MND patients clinically

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

limited to LMN symptoms / signs.[5, 6] TDP-43-immunoreactive inclusions have been detected in both the LMNs and cortical neurons of PMA patients.[7, 8] The disease course of PMA is relentlessly progressive although somewhat longer than that of ALS.[2, 4, 9, 10]

However, it is unclear whether clinically diagnosed PMA is simply a clinical phenotype of ALS in which UMN symptoms / signs are undetectable. In this study, we investigated the clinicopathological profiles of clinically diagnosed PMA patients compared with those of clinically diagnosed ALS patients using a series of consecutive adult-onset sporadic MND autopsy cases.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

METHODS

Patients and clinical evaluations

We enrolled 130 consecutive autopsied patients who were clinically diagnosed with and pathologically confirmed as suffering from sporadic, adult-onset MND at the Department of Neuropathology of the Institute for Medical Science of Aging at Aichi Medical University from January 1998 to December 2010. All of the patients had been clinically evaluated by neurological experts at the Nagoya University Hospital, the Aichi Medical University Hospital, or their affiliated hospitals. Permission to perform an autopsy and archive the brain and spinal cord for research purposes was obtained from the patient's relatives by the attending physician after death. We evaluated the clinical profiles of the included patients by retrospectively reviewing case notes written both at diagnosis and in the advanced disease stage. The disease onset was defined as the time at which the patients became aware of muscle weakness. The inclusion criteria for MND patients were as follows: older than 18 years at disease onset; no family history of ALS, PMA, progressive lateral sclerosis, inherited SMA or SBMA, or any other neurodegenerative disorder; motor neuron involvement based on neurological examination; and neuropathological evidence of neuronal loss and gliosis in the UMN and / or LMN systems, which were not due to any cerebrovascular diseases, metabolic disorders, genetic neurological disorders, inflammatory disorders, neoplasms, or traumas. We excluded 22 patients due to invalid clinical data and 1 patient with only UMN symptoms / signs throughout his disease course, and 107 patients were

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

ultimately included in this study. Based on the clinical data, we separated these 107 MND patients into two groups, namely clinical PMA and clinical ALS groups. According to a previous study,[4] clinical PMA was defined by neurological evidence of LMN involvement (decreased or diminished deep tendon reflexes and muscle atrophy) and lack of UMN symptoms / signs (increased jaw jerk, other exaggerated tendon reflexes, Babinski sign, other pathological reflexes, forced crying, and forced laughing) throughout the clinical course. Patients who exhibited motor conduction block(s) based on extensive standardized nerve conduction studies,[11] exhibited objective sensory signs (apart from mild vibration sensory disturbances in elderly patients), or had a history of diseases that may mimic MND (e.g., spinal radiculopathy, poliomyelitis, and diabetic amyotrophy) were not included in the clinical PMA group.[4] We defined clinical ALS, based on the revised El Escorial criteria, as fulfilling possible or above categories, which require UMN signs / symptoms in at least 1 region of the body.[12]

Pathological evaluations

For the pathological evaluations, we excluded one clinical PMA patient due to severe anoxic changes in the brain and 4 clinical ALS patients due to insufficient tissue material. Ultimately, we enrolled 13 clinical PMA patients and compared them with 29 clinical ALS patients who were autopsied after January 2006. In addition, 13 age-matched controls (mean age at death: 68 ± 6.91 years) were enrolled. We prepared 8-mm coronal sections of the cerebrum and 5-mm axial sections of the brainstem. The

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

tissues were fixed using 20% neutral-buffered formalin, embedded in paraffin, and sectioned at a thickness of 4.5 μm. We evaluated the sections from the precentral gyrus (4 segments from the left hemisphere), hippocampus, brainstem, and spinal cord. In all cases, the spinal cord was examined at all segment levels. Two investigators (Y. R. and M. Y.) evaluated the degeneration of the motor neuron systems and the immunohistochemical profiles of the included patients. The investigators were completely blinded to each patient's ID and the clinical diagnosis corresponding to each specimen. With respect to the degeneration of the motor neuron systems, the severity of motor neuron loss in the primary motor cortex, facial and hypoglossal nuclei, and spinal anterior horns; myelin pallor within the CST; and aggregation of macrophages within the primary motor cortex and CST were evaluated. The evaluations were performed on the most severely affected lesions and graded as none (-), mild (+), moderate (++), or severe (+++) (Fig. 1). The immunohistochemical profiles were evaluated using anti-pTDP-43 and anti-fused-in-sarcoma (FUS) antibodies in the LMN system and cerebrum. For the routine neuropathological examinations, the sections were subjected to hematoxylin-eosin (HE) or Klüver-Barrera (KB) staining. Immunohistochemistry was performed according to a standard polymer-based method using the EnVision Kit (Dako Corporation, Denmark). The primary antibodies used in this study were anti-ubiquitin (polyclonal rabbit, 1:2000; Dako, Denmark), anti-TDP-43 (polyclonal rabbit, 1:2500; ProteinTech, USA), anti-phosphorylated TDP-43 (pTDP-43 ser 409/410, polyclonal rabbit, 1:2500; CosmoBio, Japan), anti-FUS (polyclonal rabbit, 1:500; Sigma Aldrich, USA),

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

anti-alpha internexin (monoclonal mouse, 1:1000; Invitrogen, USA), anti-peripherin (polyclonal rabbit, 1:200; Millipore, USA), anti-CD68 (monoclonal mouse, 1:200; Dako), and anti-phosphorylated neurofilament (pNF, monoclonal mouse, 1:600; Dako). Diaminobenzidine (Wako, Japan) was used as the chromogen.

Quantitative analysis of large axonal fibers in the CST

To evaluate the degeneration of large axonal fibers in the CST, we calculated the density of large axonal fibers in the lateral column of the spinal cord. Specimens corresponding to the C5-6 levels, immunostained using the pNF antibody, were prepared for all of the patients and controls. The density (axonal fibers / 10,000 μm^2) of large pNF-positive axons that were more than 1 μm in diameter was automatically calculated using Lusex AP® software (Nireco, Japan) (Fig. 3a-b). The average values from 5 fields (×40 objective) were collected.

Statistical analysis

The demographic features of the PMA and ALS patients were compared using the Mann-Whitney U test for continuous variables or the Pearson chi-squared test or Fisher's exact test to assess bivariate correlations. The Kruskal-Wallis test was used for analyses between three groups, and the t t-test was used for analyses between two groups. The significance level was set at a p-value of 0.05 for

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

comparisons between two groups and 0.016 for comparisons between three groups. All of the statistical tests performed were two-sided and were conducted using the software program PASW 18.0 (IBM® SPSS®).

RESULTS

Demographic features of the registered patients

The included patients consisted of 67 men and 40 women. The mean age at disease onset was 62.7 ± 12.4 years, and the median duration from disease onset to death was 27 months (range: 2-348 months). Seventeen patients were treated with tracheostomy positive pressure ventilation (TPPV). The initial symptoms included upper-limb weakness in 40.2%, lower-limb weakness in 32.7%, bulbar symptoms in 24.3%, and respiratory symptoms in 2.8% of the included patients. Fourteen (13.1%) patients were categorized into the clinical PMA group, and 93 (86.9%) patients were classified into the clinical ALS group. The demographic features of the clinical PMA and ALS patients are presented in Table 1. In summary, no significant differences in the age at onset, male to female ratio, clinical duration (whether including or excluding the TPPV treatment period), or initial symptoms were detected between the clinical PMA and ALS groups.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

Table 1. Demographic features of clinical PMA and ALS patients

	Clinical PMA	Clinical ALS	p value
Number of patients	14	93	
Age at onset (years, mean ± S.D.)	60.8 ± 10.8	63.0 ± 12.7	0.388 [a]
Male / Female	10/4	57/36	0.563 [b]
Duration from onset to death (months) (median, range) [c]	21 (5-192)	29 (2-348)	0.764 [a]
Initial symptoms (number of patients)			
Bulbar symptoms	3 (21.4%)	23 (24.7%)	0.738 [b]
Upper-limb weakness	5 (35.7%)	38 (40.9%)	0.738 [b]
Lower-limb weakness	5 (35.7%)	30 (32.3%)	0.738 [b]
Respiratory symptoms	1 (7.1%)	2 (2.2%)	

[a] Mann-Whitney U test; [b] Fisher's exact test; [c] including the TPPV treatment period

Pathological evaluations

Degeneration in the UMN system (Fig. 2)

Loss of Betz cells in the primary motor cortex: Ten (76.9%) of the 13 clinical PMA patients exhibited a loss of Betz cells, which was severe in 3 (23.1%) of these patients. However, in 2 (15.4%)

For peer review only

of the 13 clinical PMA patients, no loss of Betz cells or gliosis in the primary motor cortex was detectable. In contrast, all of the patients diagnosed with clinical ALS exhibited a loss of Betz's cells, which was severe in 10 (34.5%) of the 29 clinical ALS patients.

Aggregation of macrophages in the primary motor cortex: Aggregation of CD-68-positive macrophages in the primary motor cortex was detected in 10 (76.9%) of the 13 clinical PMA patients. In contrast, all of the patients diagnosed with clinical ALS exhibited aggregation of macrophages in the primary motor cortex.

CST degeneration: Myelin pallor was present in 8 (61.5%) of the 13 clinical PMA patients. Aggregation of macrophages within the CST was detected in 11 (84.6%) of the 13 clinical PMA patients. In the clinical ALS group, all patients exhibited both myelin pallor and macrophage aggregation in the CST.

Degeneration in the LMN system (Fig. 2)

All of the patients diagnosed with either clinical PMA or ALS exhibited neuronal loss in the spinal anterior horns. This neuronal loss was severe in 11 (84.6%) of the 13 clinical PMA and 20 (69.0%) of the 29 clinical ALS patients. All of the patients diagnosed with clinical PMA and 27 (93.1%) of the 29 clinical ALS patients exhibited neuronal loss in the cranial nerve nuclei. This neuronal loss was severe in 6 (46.2%) of the 13 clinical PMA patients and 11 (37.9%) of the 29 clinical ALS patients. Eight

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

(61.5%) of the 13 clinical PMA patients and 24 (82.8%) of the 29 clinical ALS patients displayed Bunina bodies in the LMN system.

Immunohistochemical profiles (Fig. 2)

In 11 (84.6%) of the clinical PMA patients, we detected ubiquitin- and TDP-43-positive neuronal cytoplasmic inclusions (NCIs) in the LMN. In 8 of these patients, TDP-43-positive NCIs were also detected in the primary motor cortex. In contrast, 2 of the clinical PMA patients (15.4%) exhibited basophilic inclusion bodies in the neuronal cytoplasm, which were broadly extended throughout the central nervous system. These inclusions were positive for FUS but negative for TDP-43, alpha-internexin, and peripherin. All of the clinical ALS patients displayed ubiquitin- and TDP-43-positive NCIs in the LMN system.

Quantitative analysis of large axonal fibers in the CST (Fig. 3)

The average density of the large axonal fibers in the CST was as follows: clinical ALS, 68.3 ± 20.9 fibers / 10,000 μm^2; clinical PMA, 97.2 ± 31.5 fibers / 10,000 μm^2; and controls, 129.1 ± 6.1 fibers / 10,000 μm^2 ($p = 0.001$ between the clinical ALS and PMA groups; $p = 0.001$ between the clinical PMA and control groups; $p < 0.001$ between the clinical ALS and control groups). All of the patients diagnosed with clinical ALS exhibited lower values than the range of normal values that was obtained

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

from the controls. In contrast, the results from the clinical PMA group were widely diverse. The results from 5 (38.5%) of the 13 clinical PMA patients were within the normal range, but 8 (61.5%) of these patients exhibited lower values than the normal range. One PMA patient who had been treated with TPPV exhibited an exceptionally low value.

Pathological overview of the patients diagnosed with clinical PMA or clinical ALS

Clinical PMA: Eleven (84.6%) of the 13 clinical PMA patients displayed both UMN degeneration (either loss of Betz cells, myelin pallor, or aggregation of macrophages in the primary cortex or CST) and LMN degeneration. Nine of these patients exhibited TDP-43-positive inclusions, and the remaining 2 patients displayed FUS-positive basophilic inclusion bodies. Their CST axon densities were diverse, ranging from low values to values within the normal range that was obtained from the control subjects. In 2 (15.4%) of the 13 clinical PMA patients, degeneration in the UMN system was undetectable. These patients exhibited abundant TDP-43-positive neuronal and glial inclusions in the LMN and, occasionally, in layers II-III of the primary motor cortex and the hippocampus. Their CST axon density was within the normal range.

Clinical ALS: All 29 patients displayed a combination of UMN and LMN system degeneration and exhibited TDP-43-positive inclusions.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

Case presentation

Patient 2 in the clinical PMA group in Fig. 2

This patient was a woman who died at 75 years of age. At the age of 73 years, she presented with weakness in her left hand. A neurological examination revealed atrophy of the abductor pollicis brevis muscle in the left hand. The tendon reflexes were generally reduced, and there were no pathological reflexes. At the age of 74 years, a neurological examination revealed diffuse weakness and muscular atrophy in her upper limbs. Electromyographic analysis revealed active denervation in all four limbs and the tongue. A diagnosis of PMA was made. She died of respiratory failure 1.5 years after disease onset. No clinical UMN signs or symptoms were observed throughout her disease course. Based on a postmortem study, degeneration of the UMN system was undetectable (Fig. 4a-e), although TDP-43-positive neuronal and glial inclusions were occasionally detected in layers II-III of the motor cortex, the hippocampal dentate gyrus, and the parahippocampal gyrus (Fig. 4f, g). Severe neuronal loss, TDP-43-positive NCIs, and Bunina bodies in the LMN system were detected (Fig. 4h-j).

Patient 12 in the clinical PMA group in Fig. 2

This patient was a woman who died at 76 years of age. At the age of 74 years, she presented with weakness in her lower limbs. A neurological examination revealed muscular atrophy in her tongue, lower limbs, and hands. The tendon reflexes were reduced in her limbs, and there were no pathological

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

reflexes. Electromyographic analysis revealed active denervation in the left quadriceps femoris muscle. She died of respiratory failure 2.0 years after disease onset. No clinical UMN signs or symptoms were observed throughout her disease course. A postmortem study revealed marked degeneration in both the UMN and LMN systems (Fig. 4k-m). TDP-43-positive neuronal and glial inclusions were detected in the spinal anterior horn, cranial nerve nuclei, motor cortex, hippocampal dentate gyrus, and parahippocampal gyrus.

Patient 6 in the clinical PMA group in Fig. 2

This patient was a man who died at 62 years of age. At the age of 60 years, he presented with weakness in the upper limbs. A neurological examination revealed muscular atrophy of the shoulder girdles and arms. The tendon reflexes were generally absent, and there were no pathological reflexes. Three months following diagnosis, weakness in the respiratory muscles occurred, which was followed by management with TPPV. He died of pneumonia 1.5 years after disease onset. No clinical UMN signs or symptoms were observed throughout his disease course. A postmortem study revealed severe neuronal loss in the spinal anterior horn and mild neuronal loss in the primary motor cortex. Abundant FUS-positive basophilic inclusion bodies were detected in the neurons of the spinal anterior horn, brainstem, basal ganglia, and cerebral cortex (Fig. 4n, o). We performed genetic analysis of the FUS gene using frozen brain tissue with the consent of family members. Direct sequencing revealed no

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

mutations in any of the 15 exons or the exon/intron boundary sequences of the FUS gene in this patient.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

DISCUSSION

Our study demonstrated the clinicopathological profiles of clinical PMA and ALS patients in a consecutive autopsy series. The clinical evaluations in this study revealed rapid disease progression and short survival duration in clinical PMA patients, courses analogous to those characteristic of clinical ALS. Contrary to our results, it has been described that PMA exhibits slower progression and longer survival duration compared with ALS.[3] However, recent studies revealed that PMA follows a relentlessly progressive course and that the survival duration is not much longer than that of ALS.[2, 4, 9, 10, 13] The relatively small number of patients in our study may have contributed to the absence of significant differences in the survival durations between the clinical PMA and ALS groups.

Our pathological results indicate that clinical PMA consists of three patterns of neuropathological features: UMN and LMN degeneration with TDP-43 pathology, consistent with ALS; LMN degeneration with TDP-43 pathology but no UMN degeneration; and UMN and LMN degeneration with FUS-positive basophilic inclusion bodies. Of the clinical PMA patients, 85% exhibited degeneration in both the UMN and LMN systems, corresponding to ALS. However, the remaining 15% of the clinical PMA patients lacked any apparent degeneration in the UMN system. A previous study reported that approximately 50% of all PMA patients exhibit the presence of macrophages in the CST.[5] Another report demonstrated that degeneration of the pyramidal tract and loss of Betz cells were found in 65% and 60%, respectively, of the patients diagnosed with the PMA

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

phenotype.[6] Our results revealed that PMA patients more frequently had degeneration in the UMN system than reported in previous studies. However, in a few PMA patients, UMN degeneration remained undetectable at death.

One limitation of our study was the inability to evaluate the entire motor cortex and CST. It is controversial whether patients with no apparent degeneration of the UMN system have ALS with extremely mild UMN involvement or another pathology that is confined to the LMN system. However, the patients who lacked any apparent UMN degeneration displayed TDP-43-positive NCIs in the primary motor cortex and the hippocampal dentate gyrus, although these inclusions were limited to a very low level. A recent report described the propagation of TDP-43 pathology in ALS, which starts from the UMN and LMN systems and spreads to the antero-medial temporal lobes through the motor neuron system.[14] Based on this theory of TDP-43 propagation, the 2 patients who apparently lacked UMN degeneration could be included in the pathological spectrum of ALS.

The standard diagnostic criteria for ALS are the revised El Escorial criteria, which require a combination of UMN and LMN symptoms / signs for the diagnosis of ALS.[12] However, it is often difficult to clinically determine whether the UMN is involved, as UMN signs can be masked by severe coexisting LMN symptoms or signs.[15] Recently, several studies have demonstrated the utility of examination procedures, including transcranial magnetic stimulation, ^{1}H magnetic resonance spectroscopy, and diffusion tensor imaging, in the detection of UMN system deterioration in a subset of

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

PMA patients.[13, 15-18] Based on our results, a large subset of PMA patients may have some degree of UMN degeneration. In such patients, these radiological or electrophysiological procedures would be expected to increase the sensitivity of detection of UMN degeneration. However, our results also indicate that some of the PMA patients exhibit sparse morphological changes in the UMN system, even at death. In such patients, it may be difficult to detect UMN degeneration even using these procedures. To diagnose clinical PMA patients displaying sparse UMN degeneration as ALS in the early phase of the disease course may be a future subject of focus.

Immunohistochemically, our clinical PMA patients exhibited TDP-43 pathology or FUS-positive BIBD. TDP-43 is considered to be the major aggregated protein in PMA and ALS. In contrast, BIBD is known as a rapidly progressive, sporadic or familial MND with a younger age of onset.[19-22] However, patients with a higher age of onset have been occasionally described, such as Patient 6 in the clinical PMA group.[23-25]

In conclusion, the neuropathological profiles of clinical PMA consisted of three patterns: UMN and LMN degeneration with TDP-43 pathology, consistent with the pathological profile of ALS; LMN degeneration with TDP-43 pathology but sparing of the UMN system; and UMN and LMN degeneration with FUS-positive BIBD. One subject of future focus is how to diagnose PMA patients with few UMN degenerations as ALS in the early disease phase.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

ACKNOWLEDGEMENTS

Contributors YR and NA contributed to the conception and design of the study. All of the authors participated in the acquisition, analysis, and interpretation of the data. MY and GS drafted the manuscript. IM and WH assisted in writing and editing the manuscript.

Funding This work was supported by Grants-in-Aid from the Research Committee of CNS Degenerative Diseases of the Ministry of Health, Labor, and Welfare of Japan.

Conflicts of interest The authors declare that they have no conflicts of interest.

Ethics approval This study was approved by the ethics committees of Nagoya University and Aichi Medical University.

We give special thanks to Dr. M. Hasegawa, Department of Neuropathology and Cell Biology, Tokyo Metropolitan Institute of Medical Science, for performing the genetic analysis of the FUS genes.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

REFERENCES

1. Neumann M, Sampathu DM, Kwong LK, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 2006;314:130–3.

2. de Carvalho M, Scotto M, Swash M, et al. Clinical patterns in progressive muscular atrophy (PMA): a prospective study. Amyotroph Lateral Scler 2007;8:296-9.

3. Norris FH. Adult progressive muscular atrophy and hereditary spinal muscular atrophies. In: Vinken PJ, Bruyn GW, Klawans HL, De Jong JMBV eds. Handbook of Clinical Neurology: Diseases of the Motor System, vol. 59. Amsterdam: North-Holland Publishing Company 1991:13-34.

4. Visser J, van den Berg-Vos RM, Franssen H, et al. Disease course and prognostic factors of progressive muscular atrophy. Arch Neurol 2007;64:522-8.

5. Ince PG, Evans J, Knopp M, et al. Corticospinal tract degeneration in the progressive muscular atrophy variant of ALS. Neurology 2003;60:1252-8.

6. Brownell B, Oppenheimer DR, Hughes JT. The central nervous system in motor neurone disease. J

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

Neurol Neurosurg Psychiat 1970;33:338-57.

7. Geser F, Stein B, Partain M, et al. Motor neuron disease clinically limited to the lower motor neuron is a diffuse TDP-43 proteinopathy. Acta Neuropathol 2011;121:509-17.

8. 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.

9. Kim WK, Liu X, Sandner J, et al. Study of 962 patients indicates progressive muscular atrophy is a form of ALS. Neurology 2009;73:1686-92.

10. Van den Berg-Vos RM, Visser M, Kalmijn S, et al. A long-term prospective study of the natural course of sporadic adult-onset lower motor neuron syndromes. Arch Neurol 2009;66:751-7.

11. Koike H, Hirayama M, Yamamoto M, et al. Age associated axonal features in HNPP with 17p11.2 deletion in Japan. J Neurol Neurosurg Psychiat 2005;76:1109-14.

12. Brooks BR, Miller RG, Swash M, et al. El Escorial revisited: revised criteria for the diagnosis of

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord 2000;1:293-9.

13. Mitsumoto H, Ulug AM, Pullman SL, et al. Quantitative objective markers for upper and lower motor neuron dysfunction in ALS. Neurology 2007;68:1402-10.

14. Brettschneider J, Del Tredici K, Toledo JB, et al. Stages of pTDP-43 pathology in amyotrophic lateral sclerosis. Ann Neurol 2013;74:20-38.

15. Sach M, Winkler G, Glauche V, et al. Diffusion tensor MRI of early upper motor neuron involvement in amyotrophic lateral sclerosis. Brain 2004;127:340-50.

16. Floyd AG, Yu QP, Piboolnurak P, et al. Transcranial magnetic stimulation in ALS: utility of central motor conduction tests. Neurology 2009;72:498-504.

17. Graham JM, Papadakis N, Evans J, et al. Diffusion tensor imaging for the assessment of upper motor neuron integrity in ALS. Neurology 2004;63:2111–9.

18. Prudlo J, Bißbort C, Glass A, et al. White matter pathology in ALS and lower motor neuron ALS

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

variants: a diffusion tensor imaging study using tract-based spatial statistics. J Neurol 2012;259:1848–59.

19. Munoz DG, Neumann M, Kusaka H, et al. FUS pathology in basophilic inclusion body disease. Acta Neuropathol 2009;118:617–27

20. Neumann M, Roeber S, Kretzschmar HA, et al. Abundant FUS-immunoreactive pathology in neuronal intermediate filament inclusion disease. Acta Neuropathol 2009;118:605–16.

21. Mackenzie IRA, Ansorge O, Strong M, et al. Pathological heterogeneity in amyotrophic lateral sclerosis with FUS mutations: two distinct patterns correlating with disease severity and mutation. Acta Neuropathol 2011;122:87–98.

22. Yokota O, Tsuchiya K, Terada S, et al. Basophilic inclusion body disease and neuronal intermediate filament inclusion disease: a comparative clinicopathological study. Acta Neuropathol 2008;115:561–575.

23. Matsuoka T, Fujii N, Kondo A, et al. An autopsied case of sporadic adult-onset amyotrophic lateral

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

sclerosis with FUS-positive basophilic inclusions. Neuropathology 2011;31:71–6.

24. Sasaki S, Toi S, Shirata A, et al. Immunohistochemical and ultrastructural study of basophilic inclusions in adult-onset motor neuron disease. Acta Neuropathol 2001;102:200–6.

25. Ishihara K, Araki S, Ihori N, et al. An autopsy case of frontotemporal dementia with severe dysarthria and motor neuron disease showing numerous basophilic inclusions. Neuropathology 2006;26:447-454.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

FIGURE LEGENDS

Figure 1 Measures of degeneration in the upper motor neuron system. (A-D) Loss of Betz cells in the primary motor cortex: stage (-), the Betz cells were spared in number, and gliosis was absent (A); stage (+), mild neuronophagia and gliosis were noted (B); and stage (++), marked neuronophagia and glial proliferation were observed (C). (D-K) Aggregation of CD-68-positive macrophages in the primary motor cortex (D-G) and the corticospinal tract in the lateral column of the spinal cord (H-K): stage (-), the aggregates were absent (D, H); stage (+), the aggregates were occasionally present (E, I); stage (++), the aggregates were present at a number of 1-5 / ×100 field (F, J); and stage (+++), the aggregates were diffusely observed (G, K). L-O Myelin pallor in the CST of the lateral column of the spinal cord: stage (-), myelin pallor was not detected (L); stage (+), myelin pallor was slightly notable (M); stage (++), myelin pallor was moderate (N); and stage (+++), the CST was entirely pale. (A-C) hematoxylin-eosin staining, (D-K) anti-CD-68 immunohistochemistry, and (L-O) Klüver-Barrera staining. Scale bars: (A-G) 100 μm, (H-K) 50 μm, and (L-O) 3 mm.

Figure 2 Summary of the neuropathological findings in the included patients. The stages of the pathological changes correspond to Figure 1. Abbreviations: KB, Klüver-Barrera staining; NCI, neuronal cytoplasmic inclusion; TPPV, tracheostomy positive pressure ventilation.

For peer review only

Figure 3 Quantitative analysis of the large axonal fibers in the corticospinal tract. (A, B) To successfully evaluate the density of the large axonal fibers, phosphorylated neurofilament (pNF)-positive fibers that were more than 1 μm in diameter were automatically imaged using Lusex AP® software. The density (axonal fibers / 10,000 μm^2) of the large pNF-positive axons was automatically calculated using averaged data from 5 fields (×400). (C) There were significant differences between all pairs of clinical groups: $p = 0.001$ (*) between the clinical ALS and clinical PMA groups, $p = 0.001$ (*) between the clinical PMA and control groups, and $p < 0.001$ (**) between the clinical ALS and control groups. All patients diagnosed with clinical ALS exhibited lower values than the controls. In contrast, the results of the clinical PMA group were widely diverse, ranging from low values to values within the normal range.

Figure 4 Neuropathological profiles of the patients in the clinical PMA group. A-J correspond to Patient 2. The CST did not display myelin pallor (A), loss of large axonal fibers (B), or aggregation of macrophages (C). Additionally, in the primary motor cortex, neither loss of Betz cells (D) nor aggregation of macrophages (E) was detected. The upper layers of the primary motor cortex rarely contained phosphorylated 43-kDa TAR DNA-binding protein (pTDP-43)-positive neuronal (F) and glial (G) inclusions. The spinal anterior horn displayed severe neuronal loss (H), pTDP-43-positive skein-like inclusions (I), and Bunina bodies (J). K-M correspond to Patient 12. The CST displayed

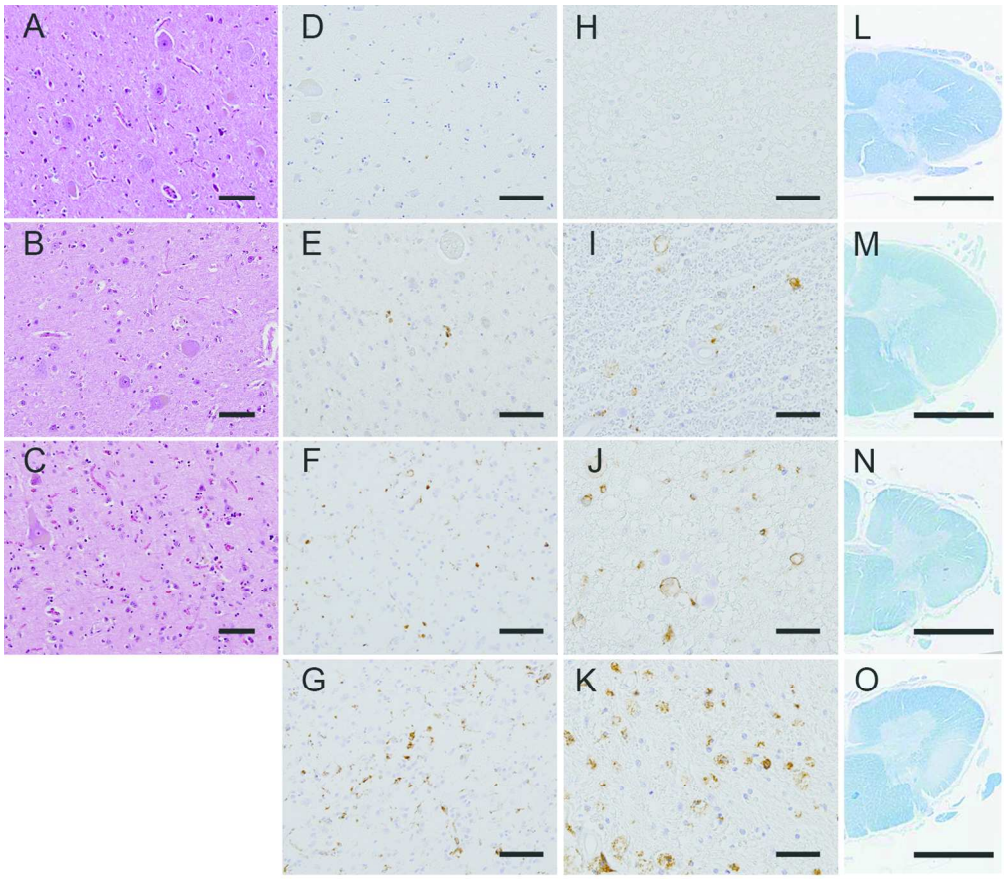
For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

myelin pallor (K) and depletion of large axonal fibers (L). Neuronophagia was often found in the primary motor cortex (M, arrows). (N-O) correspond to Patient 6. The spinal motor neurons contained basophilic inclusion bodies (N) that were positive for anti-fused-in-sarcoma (FUS) based on immunohistochemistry (O). (A, K) Klüver-Barrera staining, (B) anti-phosphorylated neurofilament immunohistochemistry, (C, E) anti-CD-68 immunohistochemistry, (D, H, J, M) Hematoxylin-eosin staining, (F, G, I) anti-pTDP-43 immunohistochemistry, and (O) anti-FUS immunohistochemistry. Scale bars: (A, K) 3 mm, (D-E) 100 µm, (C, H, M) 50 µm, (B) 20 µm, and (F, G, I, J, N, O) 10 µm.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.



128x113mm (300 x 300 DPI)

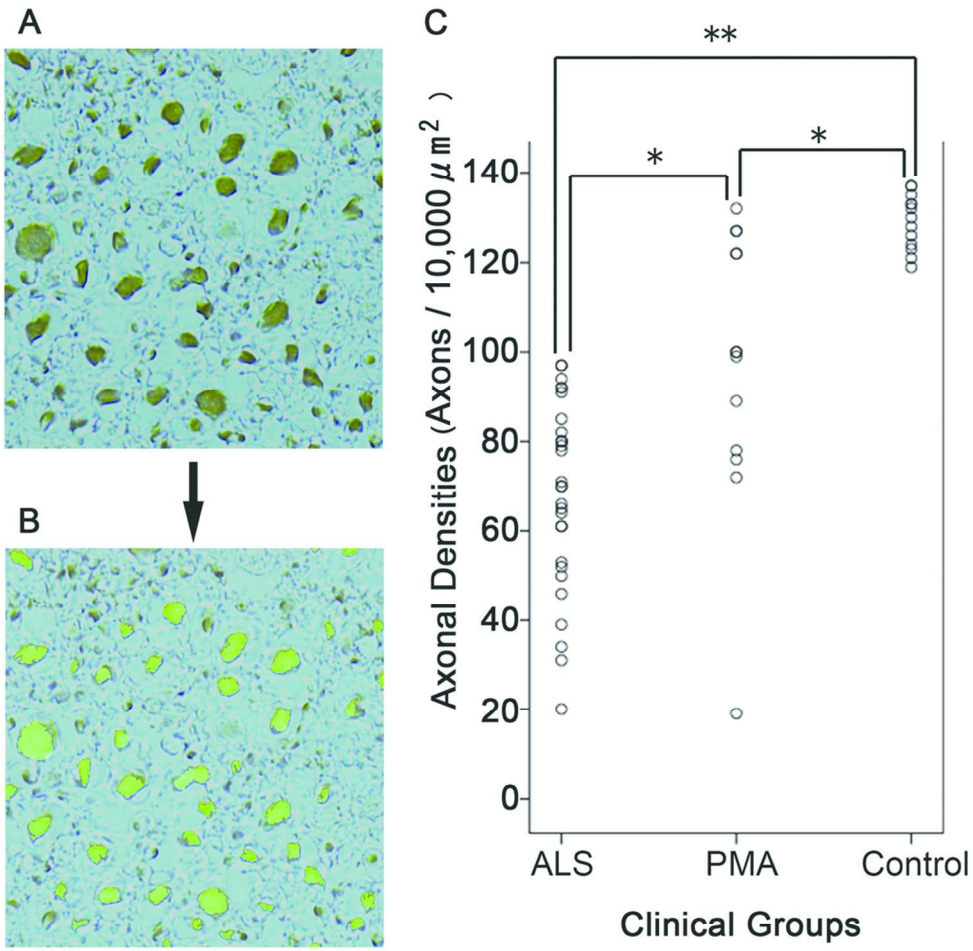
For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

102x58mm (300 x 300 DPI)

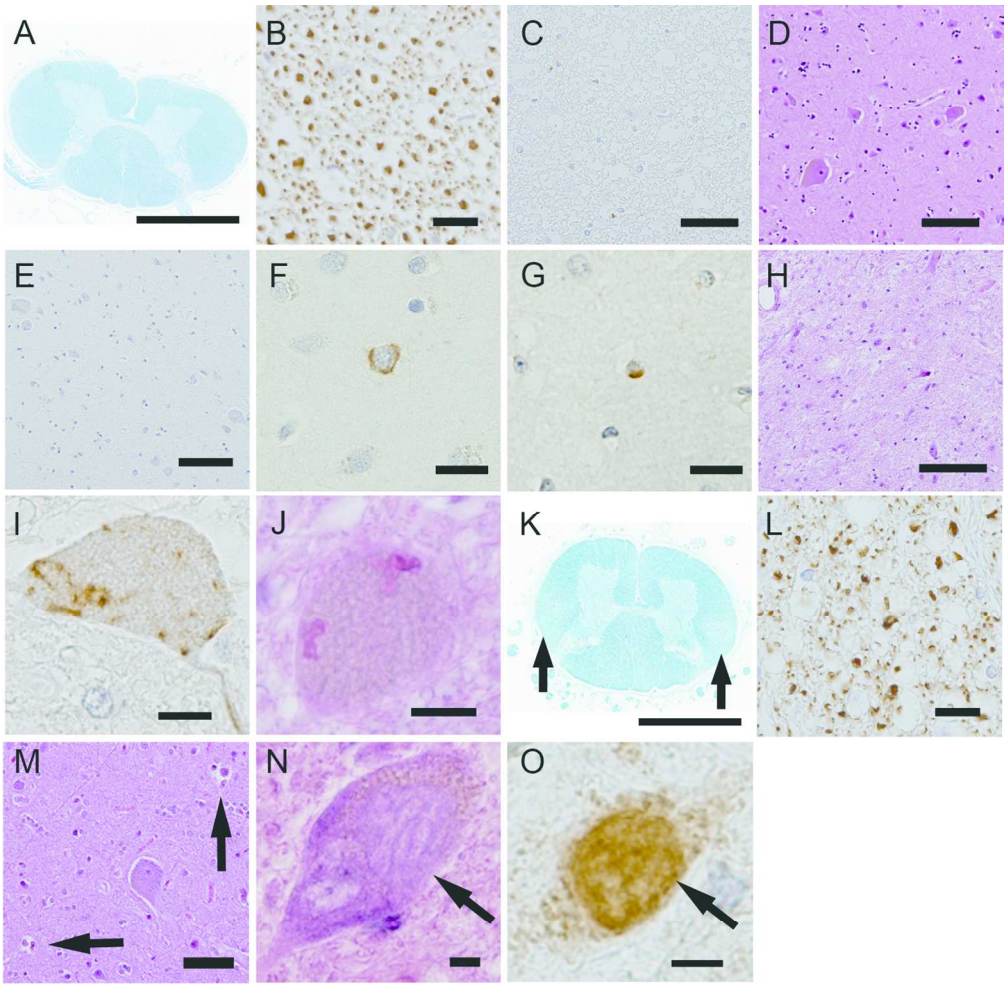
review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.



96x102mm (300 x 300 DPI)

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.



129x131mm (300 x 300 DPI)

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	√1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	√2	Explain the scientific background and rationale for the investigation being reported
Objectives	√3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	√4	Present key elements of study design early in the paper
Setting	√5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	√6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	√7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	√8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	√9	Describe any efforts to address potential sources of bias
Study size	√10	Explain how the study size was arrived at
Quantitative variables	√11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	√12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Results		
Participants	√ 13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	√ 14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	√ 15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	√ 16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	√ 17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	√ 18	Summarise key results with reference to study objectives
Limitations	√ 19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	√ 20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	√ 21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	√ 22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

2014. 1. 9

Y. Ritz

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open

Differential motor neuron involvement in progressive muscular atrophy: a comparative study with amyotrophic lateral sclerosis

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2014-005213.R1
Article Type:	Research
Date Submitted by the Author:	07-Apr-2014
Complete List of Authors:	Riku, Yuichi; Nagoya University Graduate School of Medicine, Department of Neurology Atsuta, Naoki; Nagoya University Graduate School of Medicine, Department of Neurology Yoshida, Mari; Aichi Medical University, Institute for Medical Science of Aging, Department of Neuropathology Tatsumi, Shinsui; Aichi Medical University, Institute for Medical Science of Aging, Department of Neuropathology Iwasaki, Yasushi; Aichi Medical University, Institute for Medical Science of Aging, Department of Neuropathology Mimuro, Maya; Aichi Medical University, Institute for Medical Science of Aging, Department of Neuropathology Watanabe, Hirohisa; Nagoya University Graduate School of Medicine, Department of Neurology Ito, Mizuki; Nagoya University Graduate School of Medicine, Department of Neurology Senda, Jo; Nagoya University Graduate School of Medicine, Department of Neurology Nakamura, Ryoichi; Nagoya University Graduate School of Medicine, Department of Neurology Koike, Haruki; Nagoya University Graduate School of Medicine, Department of Neurology Sobue, Gen; Nagoya University Graduate School of Medicine, Department of Neurology
Primary Subject Heading:	Neurology
Secondary Subject Heading:	Pathology
Keywords:	Neuropathology < NEUROLOGY, Motor neurone disease < NEUROLOGY, Adult neurology < NEUROLOGY

SCHOLARONE™
Manuscripts

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

Differential motor neuron involvement in progressive muscular atrophy: a comparative study with amyotrophic lateral sclerosis

Yuichi Riku[1]*, Naoki Atsuta[1]*, Mari Yoshida[2], Shinsui Tatsumi[2], Yasushi Iwasaki[2], Maya Mimuro[2], Hirohisa Watanabe[1], Mizuki Ito[1], Jo Senda[1], Ryoichi Nakamura[1], Haruki Koike[1], and Gen Sobue[1]

* These authors contributed equally to this work.

1. Department of Neurology, Nagoya University Graduate School of Medicine, Nagoya, Japan

2. Institute for Medical Science of Aging, Aichi Medical University, Aichi, Japan

Address for correspondence: Gen Sobue, MD, Department of Neurology, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan

E-mail: sobueg@med.nagoya-u.ac.jp; Fax: +81-52-744-2384; Phone: +81-52-744-2385

Word counts: Title 111 characters, Abstract 300 words, Manuscript 3598 words, Refs 20, Tables 1, and Figures 4.

Keywords: ALS, FUS, motor neuron disease, autopsy, PMA, TDP-43

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

ABSTRACT

Objective: Progressive muscular atrophy (PMA) is a clinical diagnosis characterized by progressive lower motor neuron (LMN) symptoms/signs with sporadic adult onset. It is unclear whether PMA is simply a clinical phenotype of ALS in which upper motor neuron (UMN) signs are undetectable. To elucidate the clinicopathological features of clinically diagnosed PMA patients, we studied consecutive autopsied cases.

Design: Retrospective, observational.

Setting: Autopsied patients.

Participants: We compared clinicopathological profiles of clinically diagnosed PMA and ALS using 107 consecutive autopsied patients. For the clinical analysis, 14 and 103 patients were included in clinical PMA and ALS groups, respectively. For the neuropathological evaluation, 13 clinical PMA patients and 29 clinical ALS patients were included.

Primary Outcome Measures: The clinical features, UMN and LMN degenerations, axonal density in the cortico-spinal tracts (CST), and immunohistochemical profiles.

Results: Clinically, no significant difference between the prognosis of clinical PMA and ALS groups were shown. Neuropathologically, 84.6% of clinical PMA patients displayed both UMN and LMN degeneration. In the remaining 15.4% of clinical PMA patients, neuropathological parameters that we defined as UMN degeneration were all negative or in the normal ranges. In contrast, all the clinical

For peer review only

ALS patients displayed a combination of UMN and LMN system degeneration. The CST axon densities were diverse in clinical PMA group, ranging from low values to the normal range, but consistently lower in clinical ALS group. Immunohistochemically, 85% of clinical PMA patients displayed TDP-43 pathology, while 15% displayed fused-in-sarcoma-positive basophilic inclusion bodies. All of the clinical ALS patients displayed TDP-43 pathology.

Conclusions: PMA has three neuropathological background patterns. A combination of UMN and LMN degenerations with TDP-43 pathology, consistent with ALS, is the major pathological profile. The remaining patterns have LMN degenerations with TDP-43 pathology without UMN degenerations, or a combination of UMN and LMN degenerations with FUS-positive basophilic inclusion body disease.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

ARTICLE SUMMARY

Strengths and limitations of this study

Strengths:

#1. The characteristics of motor neuron involvement in ALS or PMA were comprihensively described.

#2. The severity of upper motor neuron involvement was semi-quantitatively compared between the clinical groups, and quantitatively surrogated by axonal densities in the corticospinal tract.

#3. The pathological results clearly indicated the differences of upper motor neuron involvement between the clinical groups.

Limitations:

#1. To evaluate the entire regions in the motor cortex or corticospinal tracts is not able.

#2. We prepared formalin-fixed, paraffin-embedded tissues to quantificate axonal densities in the cortico-spinal tracts. In this protocol, the tissues can be distorted compared to the conventional fixation using glutaraldehyde and epon. The results can vary more than those from other histological techniques.

For peer review only

INTRODUCTION

Motor neuron disease (MND) constitutes a group of heterogeneous neurodegenerative diseases that are associated with progressive upper (UMN) and / or lower motor neuron (LMN) degeneration. A portion of MND cases have genetic causes; however, the majority of MND cases are sporadic and of unknown etiology. Amyotrophic lateral sclerosis (ALS) constitutes the majority of MND cases. ALS is a clinicopathological disorder that presents with progressive UMN and LMN symptoms / signs. Neuropathologically, both the UMN and LMN systems exhibit neuronal loss and gliosis, and Bunina bodies are detected in surviving neurons. Although various immunohistochemical profiles have been identified in ALS patients, 43-kDa TAR DNA-binding protein (TDP-43) is the major pathological protein in sporadic ALS.[1]

In contrast, MND that presents with LMN symptoms / signs alone occurs in several disorders, including the genetically mediated disorders spinal muscular atrophy (SMA), symmetrical axonal neuropathy, and spinal and bulbar muscular atrophy (SBMA).[2, 3] Additionally, a sporadic and adult-onset LMN disease has been referred to as progressive muscular atrophy (PMA).[3, 4] Although the revised El Escorial criteria, the standard diagnostic criteria for ALS, exclude patients who only present with LMN symptoms / signs, several studies have revealed that a subset of clinically diagnosed PMA patients exhibit the neuropathological hallmarks of ALS. Post-mortem histopathological studies have revealed cortico-spinal tract (CST) degeneration in more than half of the MND patients clinically

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

limited to LMN symptoms / signs.[5, 6] TDP-43-immunoreactive inclusions have been detected in both the LMNs and cortical neurons of PMA patients.[7, 8] The disease course of PMA is relentlessly progressive although somewhat longer than that of ALS.[2, 4, 9, 10]

However, it is unclear whether clinically diagnosed PMA is simply a clinical phenotype of ALS in which UMN symptoms / signs are undetectable. In this study, we investigated the clinicopathological profiles of clinically diagnosed PMA patients compared with those of clinically diagnosed ALS patients using a series of consecutive adult-onset sporadic MND autopsy cases.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

METHODS

Patients and clinical evaluations

We enrolled 130 consecutive autopsied patients who were clinically diagnosed with and pathologically confirmed as suffering from sporadic, adult-onset MND at the Department of Neuropathology of the Institute for Medical Science of Aging at Aichi Medical University from January 1998 to December 2010. All of the patients had been clinically evaluated by neurological experts at the Nagoya University Hospital, the Aichi Medical University Hospital, or their affiliated hospitals. Permission to perform an autopsy and archive the brain and spinal cord for research purposes was obtained from the patient's relatives by the attending physician after death. We evaluated the clinical profiles of the included patients by retrospectively reviewing case notes written both at diagnosis and in an advanced disease stage. The disease onset was defined as the time at which the patients became aware of muscle weakness. The inclusion criteria for MND patients were as follows: older than 18 years at disease onset; no family history of ALS, PMA, progressive lateral sclerosis, inherited SMA or SBMA, or any other neurodegenerative disorder; motor neuron involvement based on neurological examination; and neuropathological evidence of neuronal loss and gliosis in the UMN and / or LMN systems that were not due to any cerebrovascular diseases, metabolic disorders, genetic neurological disorders, inflammatory disorders, neoplasms, or traumas. We excluded 22 patients due to invalid clinical data and 1 patient with only UMN symptoms / signs throughout his disease course; 107 patients were

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

ultimately included in this study. Based on the clinical data, we separated these 107 MND patients into two groups, namely clinical PMA and clinical ALS groups. According to a previous study,[4] clinical PMA was defined by neurological evidence of LMN involvement (decreased or diminished deep tendon reflexes and muscle atrophy) and a lack of UMN symptoms / signs (increased jaw jerk, other exaggerated tendon reflexes, Babinski sign, other pathological reflexes, forced crying, and forced laughing) throughout the clinical course. Patients who exhibited motor conduction block(s) based on extensive standardized nerve conduction studies,[11] exhibited objective sensory signs (apart from mild vibration sensory disturbances in elderly patients), or had a history of diseases that may mimic MND (e.g., spinal radiculopathy, poliomyelitis, and diabetic amyotrophy) were not included in the clinical PMA group.[4] We defined clinical ALS, based on the revised El Escorial criteria, as fulfilling 'possible' or above categories, which require UMN signs / symptoms in at least 1 region of the body.[12]

Pathological evaluations

For the pathological evaluations, we excluded one clinical PMA patient due to severe anoxic changes in the brain and 4 clinical ALS patients due to insufficient tissue material. Ultimately, we enrolled 13 clinical PMA patients for pathological evaluations. For comparison, we enrolled 29 clinical ALS patients who were consecutively autopsied during the last 5 years of the study period (after January

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

2006). Additionally, 13 age-matched controls (mean age at death: 68 ± 6.91 years) were enrolled. We prepared 8-mm coronal sections of the cerebrum and 5-mm axial sections of the brainstem. The tissues were fixed using 20% neutral-buffered formalin, embedded in paraffin, and sectioned at a thickness of 4.5 μm. We evaluated the sections from the precentral gyrus (4 segments from the left hemisphere), hippocampus, brainstem, and spinal cord. In all cases, the spinal cord was examined at all segment levels. Two investigators (Y. R. and M. Y.) evaluated the degeneration of the motor neuron systems and the immunohistochemical profiles of the included patients. The investigators were completely blinded to each patient's ID and the clinical diagnosis corresponding to each specimen. With respect to the degeneration of the motor neuron systems, the severity of motor neuron loss in the primary motor cortex, facial and hypoglossal nuclei, and spinal anterior horns; myelin pallor within the CST; and aggregation of macrophages within the primary motor cortex and CST were evaluated. The evaluations were performed on the most severely affected lesions and graded as none (-), mild (+), moderate (++), or severe (+++) (Fig. 1). The immunohistochemical profiles were evaluated using anti-pTDP-43 and anti-fused-in-sarcoma (FUS) antibodies in the LMN system and cerebrum. For the routine neuropathological examinations, the sections were subjected to hematoxylin-eosin (HE) or Klüver-Barrera (KB) staining. Immunohistochemistry was performed according to a standard polymer-based method using the EnVision Kit (Dako, Glostrup, Denmark). The primary antibodies used in this study were anti-ubiquitin (polyclonal rabbit, 1:2000; Dako, Glostrup, Denmark),

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

anti-TDP-43 (polyclonal rabbit, 1:2500; ProteinTech, Chicago, IL, USA), anti-phosphorylated TDP-43 (pTDP-43 ser 409/410, polyclonal rabbit, 1:2500; CosmoBio, Tokyo, Japan), anti-FUS (polyclonal rabbit, 1:500; Sigma Aldrich, St. Louis, MO, USA), anti-alpha internexin (monoclonal mouse, 1:1000; Invitrogen, Carlsbad, CA, USA), anti-peripherin (polyclonal rabbit, 1:200; Millipore, Billerica, MA, USA), anti-CD68 (monoclonal mouse, 1:200; Dako, Glostrup, Denmark), anti-phosphorylated neurofilament (pNF, monoclonal mouse, 1:600; Dako, Glostrup, Denmark), and parvalbumin (polyclonal mouse, 1:1000; Sigma Aldrich, St. Louis, MO, USA). Diaminobenzidine (Wako, Osaka, Japan) was used as the chromogen.

Quantitative analysis of large axonal fibers in the CST

To evaluate the degeneration of axonal fibers in the CST, we calculated the density of axonal fibers in the lateral column of the spinal cord. Specimens corresponding to the C5-6 levels were prepared for all of the patients and 13 controls. For this assay, the paraffin-embedded spinal cords were immunostained using the anti-phosphorylated neurofilament antibody and diaminobenzidine as chromogen without additional nuclear staining to visualize only axons as brown particles. The microscopic views were binarized and automatically recognized using Luzex AP® software (Nireco, Tokyo, Japan) that was coupled to the microscope via a CCD video camera. This software automatically measured the particle counts and diameters on the binarized pictures.[13] Axonal counts were evaluated on 5 areas of 10,000

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

μm^2 (×40 objective) randomly chosen from the CST of the spinal lateral column in each patient and averaged. To validate duplicability between tests, we constructed two axon size histograms from 13 ipsilateral control samples (Supplementary File). Briefly, the variability between the test and re-test was sufficiently small to count the axons for each axon size. We constructed a histogram of axonal sizes in the CST (Fig. 3A), and the density of the large axons (axonal fibers / 10,000 μm^2) was calculated (Fig. 3B-C) for the PMA and ALS patients and control samples.

Statistical analysis

The demographic features of the PMA and ALS patients were compared using the Mann-Whitney U test for continuous variables or the Pearson's chi-squared test or Fisher's exact test to assess bivariate correlations. The Kruskal-Wallis test was used for analyses between three groups, and the t-test was used for analyses between two groups. The significance level was set at a p-value of 0.05 for comparisons between two groups and 0.016 for comparisons between three groups. All of the statistical tests performed were two-sided and were conducted using the software program PASW 18.0 (IBM® SPSS®).

RESULTS

Demographic features of the registered patients

For peer review only

The included patients consisted of 67 men and 40 women. The mean age at disease onset was 62.7 ± 12.4 years, and the median duration from disease onset to death was 27 months (range: 2-348 months). Seventeen patients were treated with tracheostomy positive-pressure ventilation (TPPV). The initial symptoms included upper-limb weakness in 40.2%, lower-limb weakness in 32.7%, bulbar symptoms in 24.3%, and respiratory symptoms in 2.8% of the included patients. Fourteen (13.1%) patients were categorized into the clinical PMA group, and 93 (86.9%) patients were classified into the clinical ALS group. With regard to clinical diagnosis, 10 (71.4%) of 14 clinical PMA patients and 88 (94.6%) of 93 clinical ALS patients were correctly diagnosed as PMA or ALS by the first referred physicians. However, 1clinical PMA patient and 4 of clinical ALS patients were initially diagnosed as having cervical or lumbar canal stenosis based on focal weakness restricted to one upper or lower limb and canal stenosis on MRI. One of the clinical PMA patients was initially diagnosed as having carpal tunnel syndrome based on weakness restricted to distal area of the median nerve in the right hand. One of clinical PMA was diagnosed as having polyradiculopathy because the cauda equina was slightly enhanced on gadolinium-enhanced MRI. One of clinical PMA patients was initially diagnosed as having myositis based on myalgia and slight lymphatic infiltration on a muscle biopsy. One of clinical ALS patients was initially diagnosed as having parkinsonian syndrome because the patient showed bradykinesia due to marked rigo-spasticity in the limbs. The demographic features of the clinical PMA and ALS patients are presented in Table 1. In summary, no significant differences in the age at onset,

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

male to female ratio, clinical duration (whether including or excluding the TPPV treatment period), or initial symptoms were detected between the clinical PMA and ALS groups.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

Table 1. Demographic features of clinical PMA and ALS patients

	Clinical PMA	Clinical ALS	p value
Number of patients	14	93	
Age at onset (years, mean ± S.D.)	60.8 ± 10.8	63.0 ± 12.7	0.388 [a]
Male / Female	10/4	57/36	0.563 [b]
Duration from onset to death (months) (median, range) [c]	21 (5-192)	29 (2-348)	0.764 [a]
Initial symptoms (number of patients)			
Bulbar symptoms	3 (21.4%)	23 (24.7%)	0.738 [b]
Upper-limb weakness	5 (35.7%)	38 (40.9%)	0.738 [b]
Lower-limb weakness	5 (35.7%)	30 (32.3%)	0.738 [b]
Respiratory symptoms	1 (7.1%)	2 (2.2%)	

[a] Mann-Whitney U test; [b] Fisher's exact test; [c] including the TPPV treatment period

Pathological evaluations

Degeneration in the UMN system (Fig. 2)

Loss of Betz cells in the primary motor cortex: Ten (76.9%) of the 13 clinical PMA patients exhibited a loss of Betz cells, which was severe in 3 (23.1%) of these patients. However, in 2 (15.4%)

For peer review only

of the 13 clinical PMA patients, no loss of Betz cells or gliosis in the primary motor cortex was detectable. In contrast, all of the patients diagnosed with clinical ALS exhibited a loss of Betz's cells, which was severe in 10 (34.5%) of the 29 clinical ALS patients. There was no significant difference in the severity of this pathological change between the clinical groups.

Aggregation of macrophages in the primary motor cortex: The aggregation of CD-68-positive macrophages in the primary motor cortex was detected in 10 (76.9%) of the 13 clinical PMA patients. In contrast, all of the patients diagnosed with clinical ALS exhibited the aggregation of macrophages in the primary motor cortex. When comparing the clinical groups, this pathological change was significantly more severe in clinical ALS than clinical PMA ($p = 0.048$).

CST degeneration: Myelin pallor was present in 8 (61.5%) of the 13 clinical PMA patients. The aggregation of macrophages within the CST was detected in 11 (84.6%) of the 13 clinical PMA patients. In the clinical ALS group, all patients exhibited both myelin pallor and macrophage aggregation in the CST. When comparing the clinical groups, this pathological change was significantly more severe in clinical ALS than clinical PMA ($p = 0.004$).

Degeneration in the LMN system (Fig. 2)

All of the patients diagnosed with either clinical PMA or ALS exhibited neuronal loss in the spinal anterior horns. This neuronal loss was severe in 11 (84.6%) of the 13 clinical PMA and 20 (69.0%) of

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

the 29 clinical ALS patients. All of the patients diagnosed with clinical PMA and 27 (93.1%) of the 29 clinical ALS patients exhibited neuronal loss in the cranial nerve nuclei. This neuronal loss was severe in 6 (46.2%) of the 13 clinical PMA patients and 11 (37.9%) of the 29 clinical ALS patients. When comparing the clinical groups, there was no significant difference in the severity of LMN loss. Eight (61.5%) of the 13 clinical PMA patients and 24 (82.8%) of the 29 clinical ALS patients displayed Bunina bodies in the LMN system.

Immunohistochemical profiles (Fig. 2)

In 11 (84.6%) of the clinical PMA patients, we detected ubiquitin- and TDP-43-positive neuronal cytoplasmic inclusions (NCIs) in the LMN. In 8 of these patients, TDP-43-positive NCIs were also detected in the primary motor cortex. All of the clinical ALS patients displayed ubiquitin- and TDP-43-positive NCIs in the LMN system. Moreover, TDP-43-positive glial cytoplasmic inclusions were observed in the spinal anterior horn and primary motor cortex in all of the TDP-43-positive patients of the clinical ALS and PMA groups. In contrast, 2 of the clinical PMA patients (15.4%) exhibited basophilic inclusion bodies in the neuronal cytoplasm, which were broadly extended throughout the central nervous system. These inclusions were positive for FUS but negative for TDP-43, alpha-internexin, and peripherin.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

Quantitative analysis of large axonal fibers in the CST (Fig. 3)

The histogram of axonal sizes revealed that the percentage of axons that were greater than 1 μm in diameter were smaller in ALS (18.5%) and PMA (23.9%) than in controls (32.3%), resulting in a relative increase of the percentage of smaller axons. Then, we measured the densities of large axons densities that were greater than 1 μm in diameter. The average densities were as follows: clinical ALS, 68.3 ± 20.9 fibers / 10,000 μm^2; clinical PMA, 97.2 ± 31.5 fibers / 10,000 μm^2; and controls, 129.1 ± 6.1 fibers / 10,000 μm^2 ($p = 0.001$ between the clinical ALS and PMA groups; $p = 0.001$ between the clinical PMA and control groups; $p < 0.001$ between the clinical ALS and control groups). All of the patients diagnosed with clinical ALS exhibited lower values than the range of normal values that was obtained from the controls. In contrast, the results from the clinical PMA group were widely diverse. The results from 5 (38.5%) of the 13 clinical PMA patients were within the normal range, but 8 (61.5%) of these patients exhibited lower values than the normal range. One PMA patient who had been treated with TPPV exhibited an exceptionally low value.

Pathological overview of the patients diagnosed with clinical PMA or clinical ALS

Clinical PMA: Eleven (84.6%) of the 13 clinical PMA patients displayed both UMN degeneration (either the loss of Betz cells, myelin pallor, or the aggregation of macrophages in the primary cortex or CST) and LMN degeneration. Nine of these patients exhibited TDP-43-positive inclusions, and the

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

remaining 2 patients displayed FUS-positive basophilic inclusion bodies. Their large CST axon densities were diverse, ranging from low values to values within the normal range that was obtained from the control subjects. In 2 (15.4%) of the 13 clinical PMA patients, neuropathological parameters that we defined as the UMN system degeneration were all negative. Their large CST axon density was within the normal range. These 2 patients exhibited abundant TDP-43-positive neuronal and glial inclusions in the LMN and, occasionally, in layers II-III of the primary motor cortex and the hippocampus. The pathological findings from the representative patients are shown in Fig. 4.

Clinical ALS: All 29 patients displayed a combination of UMN and LMN system degeneration and exhibited TDP-43-positive inclusions.

Additionally, of the respirator-managed patients, 3 patients (Patient 13 of clinical PMA and Patients 27 and 28 of clinical ALS) showed diffusely extended neuronal loss, gliosis, and TDP-43 pathology beyond the motor neuron systems, which involved all layers of the cerebral neocortices, the striatum, the thalamus, the cerebellar dentate nucleus, and the non-motor nuclei in the brainstem, including the substantia nigra, the red nucleus, the periaqueductal gray matter, the inferior olivary nucleus, and the reticular formation.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

DISCUSSION

Our study demonstrated the clinicopathological profiles of clinical PMA and ALS patients in a consecutive autopsy series. The clinical evaluations in this study revealed rapid disease progression and short survival duration in clinical PMA patients, which are analogous courses to those that are characteristic of clinical ALS. Contrary to our results, it has been described that PMA exhibits slower progression and longer survival duration compared with ALS.[3] However, recent studies revealed that PMA follows a relentlessly progressive course and that the survival duration is not much longer than that of ALS.[2, 4, 9, 10, 14] The relatively small number of patients in our study may have contributed to the absence of significant differences in the survival durations between the clinical PMA and ALS groups.

Our pathological results indicate that, of the clinical PMA patients, 85% exhibited degeneration in both the UMN and LMN systems, which corresponds with ALS. However, the remaining 15% of the clinical PMA patients lacked any apparent degeneration in the UMN system. A previous study reported that approximately 50% of all PMA patients exhibit macrophages in the CST.[5] Another report demonstrated the degeneration of the pyramidal tract and loss of Betz cells in 65% and 60%, respectively, of the patients diagnosed with the PMA phenotype.[6] Our results revealed that PMA patients more frequently had degeneration in the UMN system than those reported in previous studies; however, in a few PMA patients, the UMN degeneration remained undetectable at

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

death. Our pathological results revealed differential UMN involvement between PMA patients and indicated that PMA and ALS are in a continuous pathological entity. Regarding immunohistochemical aspects, several studies have revealed that TDP-43 pathology is commonly observed in the cerebral cortices or the subcortical gray matters of PMA patients.[7,8] In our results, TDP-43-positive neuronal or glial inclusions in the motor cortices or hippocampus was common both in clinical ALS and PMA groups and was found even in patients apparently lacking UMN degenerative changes. A recent report described the propagation of TDP-43 pathology in ALS, which starts from the UMN and LMN systems and spreads to the antero-medial temporal lobes through the motor neuron system.[15] Based on this theory of TDP-43 propagation, TDP-43 pathology beyond the LMN system in PMA patients may support the pathological continuity between these two clinical phenotypes.

The standard diagnostic criteria for ALS are the revised El Escorial criteria, which require a combination of UMN and LMN symptoms / signs for the diagnosis of ALS.[12] However, it is often difficult to clinically determine whether the UMN is involved,[16] which sometimes results in diagnostic difficulty. In our patient series, only 71.4% of the clinical PMA patients were correctly diagnosed by the first referred physicians, although 94.6% of the clinical ALS patients were. Recently, several studies have demonstrated the utility of radiological procedures, including transcranial magnetic stimulation, ^{1}H magnetic resonance spectroscopy, and diffusion tensor imaging, in the detection of UMN system deterioration in a subset of PMA patients.[14, 17-20] Based on our results, a

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

large subset of PMA patients may have some degree of UMN degeneration. In such patients, these radiological or electrophysiological procedures would be expected to increase the sensitivity of detection of UMN degeneration. However, our results also indicate that some of the PMA patients exhibit sparse morphological changes in the UMN system, even at death. It may be difficult to detect UMN degeneration using these procedures in such patients. To diagnose clinical PMA patients displaying sparse UMN degeneration as ALS in the early phase of the disease course may be a future subject of focus.

A limitation of our study was the inability to evaluate the entire motor cortex and CST, and it is controversial whether patients apparently intact UMN systems actually lack or have extremely mild UMN involvement. Another methodological limitation is that we evaluated axonal sizes and densities using neutral formalin-fixed, paraffin embedded specimens. The tissues may be somewhat distorted when compared with conventional nerve fixation using glutaraldehyde followed by epon embedding. Our methods were considered to be appropriate to assess the proportional changes in sizes of pyramidal axons, but the absolute values of axonal diameters can vary from those that have have been obtained using other histological techniques.[13]

In summary, 84.6% of clinical PMA patients displayed both UMN and LMN degeneration, which is consistent with the pathological profiles of ALS. In 15.4% of the clinical PMA patients, degeneration in the UMN system was undetectable. The large axon density in the CST varied from low

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

values to a normal range. In contrast, all of the clinical ALS patients displayed a combination of UMN and LMN system degeneration and significantly reduced large axon density in the CST.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

ACKNOWLEDGEMENTS

Contributors YR and NA contributed to the conception and design of the study. All of the authors participated in the acquisition, analysis, and interpretation of the data. MY and GS drafted the manuscript. IM and WH assisted in writing and editing the manuscript.

Funding This work was supported by Grants-in-Aid from the Research Committee of CNS Degenerative Diseases of the Ministry of Health, Labor, and Welfare of Japan.

Conflicts of interest The authors declare that they have no conflicts of interest.

Ethics approval This study was approved by the ethics committees of Nagoya University and Aichi Medical University.

We give special thanks to Dr. M. Hasegawa, Department of Neuropathology and Cell Biology, Tokyo Metropolitan Institute of Medical Science, for performing the genetic analysis of the FUS genes.

Data sharing No additional data available.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

REFERENCES

1. Neumann M, Sampathu DM, Kwong LK, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 2006;314:130–3.

2. de Carvalho M, Scotto M, Swash M, et al. Clinical patterns in progressive muscular atrophy (PMA): a prospective study. Amyotroph Lateral Scler 2007;8:296-9.

3. Norris FH. Adult progressive muscular atrophy and hereditary spinal muscular atrophies. In: Vinken PJ, Bruyn GW, Klawans HL, De Jong JMBV eds. Handbook of Clinical Neurology: Diseases of the Motor System, vol. 59. Amsterdam: North-Holland Publishing Company 1991:13-34.

4. Visser J, van den Berg-Vos RM, Franssen H, et al. Disease course and prognostic factors of progressive muscular atrophy. Arch Neurol 2007;64:522-8.

5. Ince PG, Evans J, Knopp M, et al. Corticospinal tract degeneration in the progressive muscular atrophy variant of ALS. Neurology 2003;60:1252-8.

6. Brownell B, Oppenheimer DR, Hughes JT. The central nervous system in motor neurone disease. J

For peer review only

Neurol Neurosurg Psychiat 1970;33:338-57.

7. Geser F, Stein B, Partain M, et al. Motor neuron disease clinically limited to the lower motor neuron is a diffuse TDP-43 proteinopathy. Acta Neuropathol 2011;121:509-17.

8. 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.

9. Kim WK, Liu X, Sandner J, et al. Study of 962 patients indicates progressive muscular atrophy is a form of ALS. Neurology 2009;73:1686-92.

10. Van den Berg-Vos RM, Visser M, Kalmijn S, et al. A long-term prospective study of the natural course of sporadic adult-onset lower motor neuron syndromes. Arch Neurol 2009;66:751-7.

11. Koike H, Hirayama M, Yamamoto M, et al. Age associated axonal features in HNPP with 17p11.2 deletion in Japan. J Neurol Neurosurg Psychiat 2005;76:1109-14.

12. Brooks BR, Miller RG, Swash M, et al. El Escorial revisited: revised criteria for the diagnosis of

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord 2000;1:293-9.

13. Sobue G, Hashizume Y, Mitsuma T, et al. Size-dependent myelinated fiber loss in the corticospinal tract in Shy-Drager syndrome and amyotrophic lateral sclerosis. Neurology 1987;37:529-32.

14. Mitsumoto H, Ulug AM, Pullman SL, et al. Quantitative objective markers for upper and lower motor neuron dysfunction in ALS. Neurology 2007;68:1402-10.

15. Brettschneider J, Del Tredici K, Toledo JB, et al. Stages of pTDP-43 pathology in amyotrophic lateral sclerosis. Ann Neurol 2013;74:20-38.

16. Swash M. Why are upper motor neuron signs difficult to elicit in amyotrophic lateral sclerosis? J Neurol Neurosurg Psychiat 2012;83:659-62.

17. Sach M, Winkler G, Glauche V, et al. Diffusion tensor MRI of early upper motor neuron involvement in amyotrophic lateral sclerosis. Brain 2004;127:340-50.

18. Vucic S, Ziemann U, Eisen A, et al. Transcranial magnetic stimulation and amyotrophic lateral

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

sclerosis: pathophysiological insights. J Neurol Neurosurg Psychiatry 2013;84:1161-70.

19. Graham JM, Papadakis N, Evans J, et al. Diffusion tensor imaging for the assessment of upper motor neuron integrity in ALS. Neurology 2004;63:2111–9.

20. Prudlo J, Bißbort C, Glass A, et al. White matter pathology in ALS and lower motor neuron ALS variants: a diffusion tensor imaging study using tract-based spatial statistics. J Neurol 2012;259:1848–59.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

FIGURE LEGENDS

Figure 1 Measures of degeneration in the upper motor neuron system. (A-D) Loss of Betz cells in the primary motor cortex: stage (-), the Betz cells were spared in number, and gliosis was absent (A); stage (+), mild neuronophagia and gliosis were noted (B); and stage (++), marked neuronophagia and glial proliferation were observed (C). (D-K) Aggregation of CD-68-positive macrophages in the primary motor cortex (D-G) and the corticospinal tract in the lateral column of the spinal cord (H-K): stage (-), the aggregates were absent (D, H); stage (+), the aggregates were occasionally present (E, I); stage (++), the aggregates were present at a number of 1-5 / ×100 field (F, J); and stage (+++), the aggregates were diffusely observed (G, K). L-O Myelin pallor in the CST of the lateral column of the spinal cord: stage (-), myelin pallor was not detected (L); stage (+), myelin pallor was slightly notable (M); stage (++), myelin pallor was moderate (N); and stage (+++), the CST was entirely pale. (A-C) hematoxylin-eosin staining, (D-K) anti-CD-68 immunohistochemistry, and (L-O) Klüver-Barrera staining. Scale bars: (A-G) 100 μm, (H-K) 50 μm, and (L-O) 3 mm.

Figure 2 Summary of the neuropathological findings in the included patients. The stages of the pathological changes correspond to those in Figure 1. Pathological changes between the clinical groups were compared using Pearson's chi-squared test. Abbreviations: GCI, glial cytoplasmic inclusions; KB, Klüver-Barrera staining; NCI, neuronal cytoplasmic inclusion; TPPV, tracheostomy positive pressure

For peer review only

ventilation.

Figure 3 Quantitative analysis of the axonal fibers in the corticospinal tract. (A) Phosphorylated neurofilament (pNF)-positive fibers were automatically binarized using Luzex AP® software. The density of pNF-positive axons (particles / 10,000 μm ^{2}) was automatically calculated using averaged data from 5 fields (×400). The histogram of axonal sizes revealed that the percentages of axons that were more than 1 μm in diameter were smaller in ALS and PMA than in controls. (B) The large axonal fibers more than 1 μm in diameter were automatically recognized, binarized, and counted using the software to successfully evaluate the axonal density. (C) There were significant differences in the densities of axons that were more than 1 μm in diameter between all pairs of clinical groups: $p = 0.001$ (*) between the clinical ALS and clinical PMA groups, $p = 0.001$ (*) between the clinical PMA and control groups, and $p < 0.001$ (**) between the clinical ALS and control groups. All patients diagnosed with clinical ALS exhibited lower values than the controls. In contrast, the results of the clinical PMA group were widely diverse, ranging from low values to values within the normal range.

Figure 4 Neuropathological profiles of the patients in the clinical PMA group. (A) - (J) correspond to Patient 2. The CST did not display myelin pallor (A), loss of large axonal fibers (B), or aggregation of macrophages (C). Additionally, in the primary motor cortex, neither the loss of Betz cells (D) nor

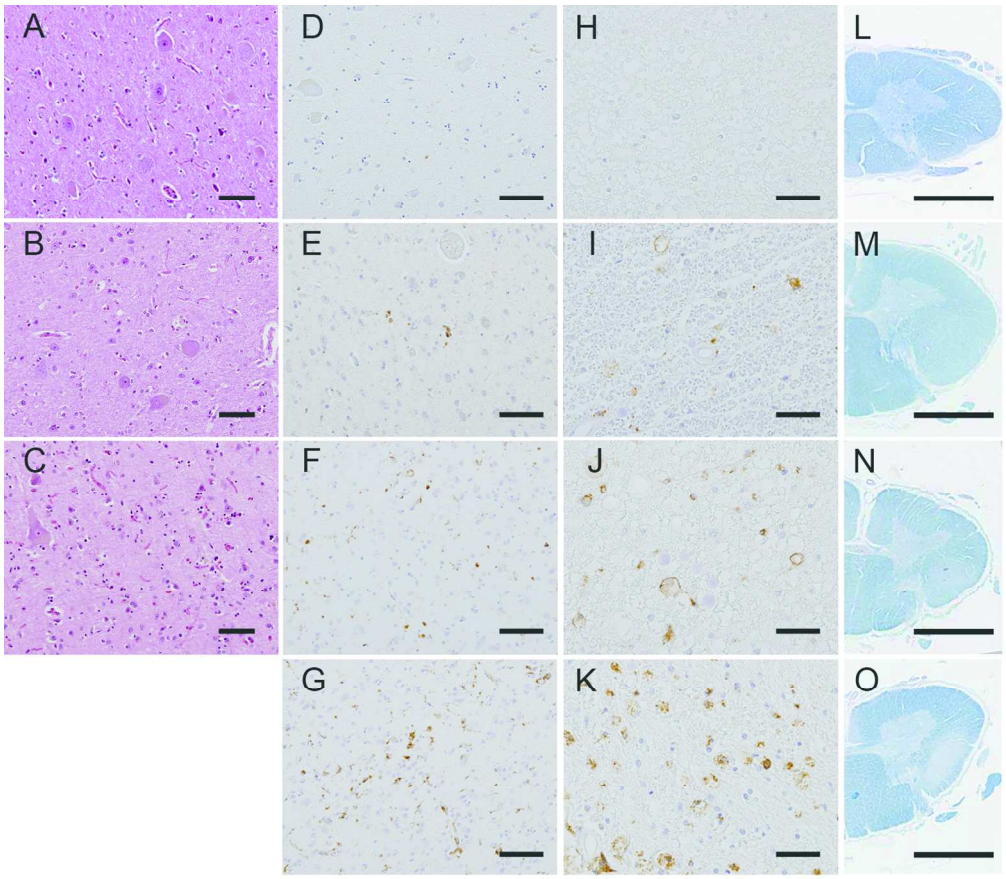
For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

aggregation of macrophages (E) was detected. The upper layers of the primary motor cortex rarely contained phosphorylated 43-kDa TAR DNA-binding protein (pTDP-43)-positive neuronal (F) and glial (G) inclusions. The spinal anterior horn displayed severe neuronal loss (H), pTDP-43-positive skein-like inclusions (I), and Bunina bodies (J). (K) – (M) correspond to Patient 12. The CST displayed myelin pallor (K) and the depletion of large axonal fibers (L). Neuronophagia was often found in the primary motor cortex (M, arrows). (N) – (O) correspond to Patient 6. The spinal motor neurons contained basophilic inclusion bodies (N) that were positive for anti-fused-in-sarcoma (FUS) based on immunohistochemistry (O). (A, K) Klüver-Barrera staining, (B) anti-phosphorylated neurofilament immunohistochemistry, (C, E) anti-CD-68 immunohistochemistry, (D, H, J, M) Hematoxylin-eosin staining, (F, G, I) anti-pTDP-43 immunohistochemistry, and (O) anti-FUS immunohistochemistry. Scale bars: (A, K) 3 mm, (D-E) 100 μm, (C, H, M) 50 μm, (B) 20 μm, and (F, G, I, J, N, O) 10 μm.

For peer review only

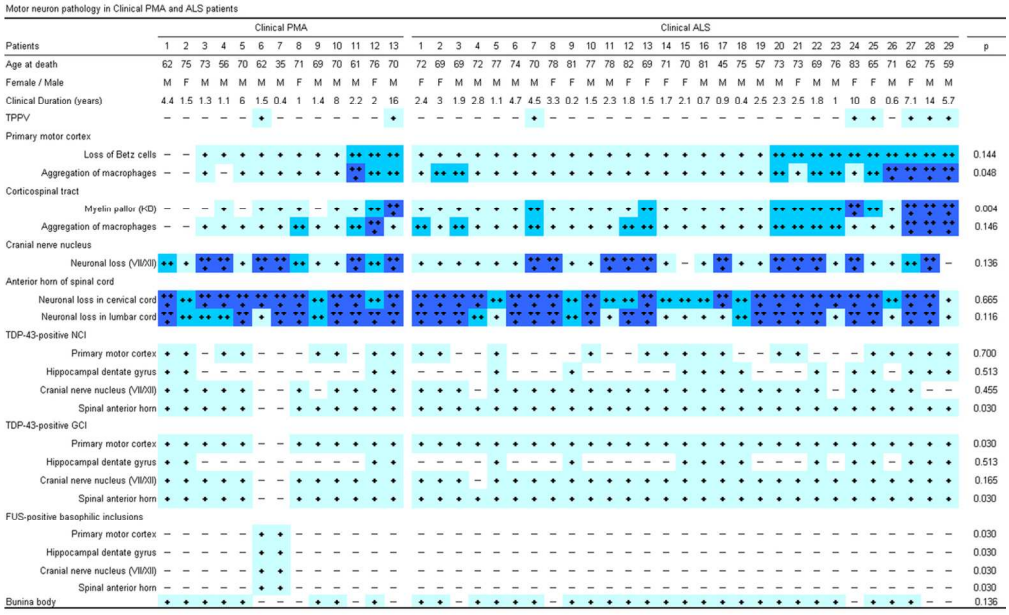
BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.



128x113mm (300 x 300 DPI)

For peer review only

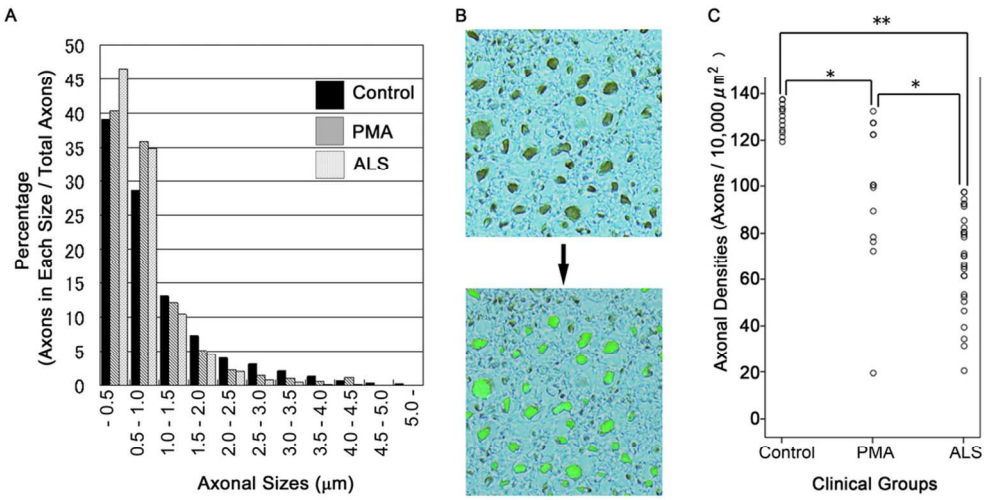
BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.



117x73mm (300 x 300 DPI)

For peer review only

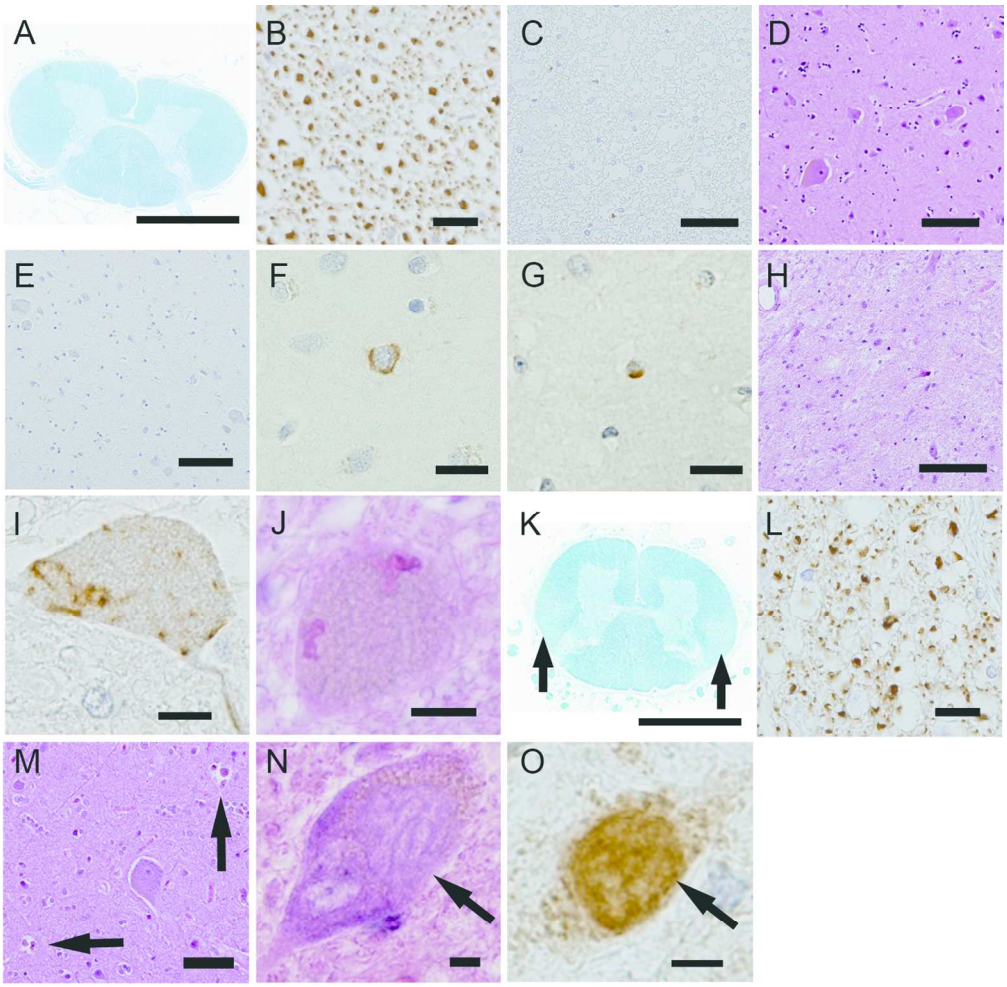
BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.



96x53mm (300 x 300 DPI)

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.



129x131mm (300 x 300 DPI)

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

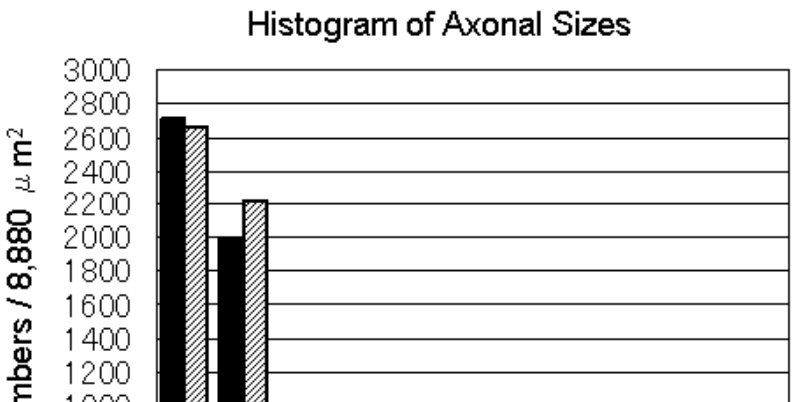


Figure. Histogram of axonal sizes in control materials.

The axonal sizes of 13 control materials are shown. The axonal count was measured twice on the ipsilateral cortico-spinal tract in the lateral column. The results from first test and re-test are described as black and slashed bars, respectively. The total axonal counts were 6928 in the first test and 6847 in the re-test.

For peer review only

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	√1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	√2	Explain the scientific background and rationale for the investigation being reported
Objectives	√3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	√4	Present key elements of study design early in the paper
Setting	√5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	√6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	√7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	√8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	√9	Describe any efforts to address potential sources of bias
Study size	√10	Explain how the study size was arrived at
Quantitative variables	√11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	√12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Results		
Participants	√ 13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	√ 14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	√ 15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	√ 16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	√ 17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	√ 18	Summarise key results with reference to study objectives
Limitations	√ 19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	√ 20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	√ 21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	√ 22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

2014. 1. 9

Y. Ritz

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Differential motor neuron involvement in progressive muscular atrophy: a comparative study with amyotrophic lateral sclerosis

Yuichi Riku[1]*, Naoki Atsuta[1]*, Mari Yoshida[2], Shinsui Tatsumi[2], Yasushi Iwasaki[2], Maya Mimuro[2], Hirohisa Watanabe[1], Mizuki Ito[1], Jo Senda[1], Ryoichi Nakamura[1], Haruki Koike[1], and Gen Sobue[1]

* These authors contributed equally to this work.

1. Department of Neurology, Nagoya University Graduate School of Medicine, Nagoya, Japan

2. Institute for Medical Science of Aging, Aichi Medical University, Aichi, Japan

Address for correspondence: Gen Sobue, MD, Department of Neurology, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan

E-mail: sobueg@med.nagoya-u.ac.jp; Fax: +81-52-744-2384; Phone: +81-52-744-2385

Word counts: Title 111 characters, Abstract 300~~249~~ words, Manuscript 3598~~376~~ words, Refs 20~~5~~, Tables 1, and Figures 4.

Keywords: ALS, FUS, motor neuron disease, autopsy, PMA, TDP-43

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

ABSTRACT

Objective: Progressive muscular atrophy (PMA) is a clinical diagnosis characterized by progressive lower motor neuron (LMN) symptoms/signs with sporadic adult onset. ~~Several studies have indicated that PMA often exhibits pathological features of amyotrophic lateral sclerosis (ALS). However, I~~it is unclear whether PMA is simply a clinical phenotype of ALS in which upper motor neuron (UMN) signs are undetectable. To elucidate the clinicopathological features of clinically diagnosed PMA patients, we studied consecutive autopsied cases.

Design: Retrospective, observational.

Setting: Autopsied patients.

Participants: ~~Methods~~: We compared clinicopathological profiles of clinically diagnosed PMA and ALS using 107 consecutive autopsied patients. For the clinical analysis, 14 and 103 patients were included in clinical PMA and ALS groups, respectively. For the neuropathological evaluation, 13 clinical PMA patients and 29 clinical ALS patients were included.

Primary Outcome Measures: The clinical features, UMN and LMN degenerations, axonal density in the cortico-spinal tracts (CST), and immunohistochemical profiles.~~Degeneration in the UMN and LMN systems, axonal density in the cortico-spinal tracts, and immunohistochemical profiles were evaluated.~~

Results: Clinically, no significant difference between the prognosis of clinical PMA and ALS groups were shown. Neuropathologically, 84.6% of clinical PMA patients displayed both UMN and LMN

Formatted: Font: Bold

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

degeneration. In the remaining 15.4% of clinical PMA patients, neuropathological parameters that we defined as UMN degeneration were all negative or in the normal ranges. In contrast, all the clinical ALS patients displayed a combination of UMN and LMN system degeneration. The CST axon densities were diverse in clinical PMA group, ranging from low values to the normal range, but consistently lower in clinical ALS group. Immunohistochemically, 85% of clinical PMA patients displayed TDP-43 pathology, while 15% displayed fused-in-sarcoma-positive basophilic inclusion bodies. All of the clinical ALS patients displayed TDP-43 pathology.~~On the clinical evaluation, no significant difference between the prognosis of clinical PMA and ALS groups. On the pathological evaluation, 85% of clinical PMA patients showed degeneration in the UMN system. The large axon density in the cortico-spinal tracts of clinical PMA patients was higher on average than that of clinical ALS patients (p = 0.001). Immunohistochemically, 85% of clinical PMA patients displayed TDP-43-positive inclusions, while 15% displayed fused-in-sarcoma (FUS)-positive basophilic inclusion bodies. All of the clinical ALS patients displayed both UMN and LMN degeneration and TDP-43-positive inclusions.~~

Conclusions: PMA has three neuropathological background patterns. A combination of UMN and LMN degenerations with TDP-43 pathology, consistent with ALS, is the major pathological profile. The remaining patterns have LMN degenerations with TDP-43 pathology without UMN degenerations, or a combination of UMN and LMN degenerations with FUS-positive basophilic inclusion body

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

For peer review only

disease. PMA consists of three neuropathological patterns: UMN and LMN degeneration with TDP-43-pathology, consistent with ALS; LMN degeneration with TDP-43-pathology but sparing of the UMN system; and UMN and LMN degeneration with FUS-positive basophilic inclusion bodies.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

ARTICLE SUMMARY

Strengths and limitations of this study

Strengths:

#1. The characteristics of motor neuron involvement in ALS or PMA were comprihensively described.

#2. The severity of upper motor neuron involvement was semi-quantitatively compared between the clinical groups, and quantitatively surrogated by axonal densities in the corticospinal tract.

#3. The pathological results clearly indicated the differences of upper motor neuron involvement between the clinical groups.

Limitations:

#1. To evaluate the entire regions in the motor cortex or corticospinal tracts is not able.

#2. We prepared formalin-fixed, paraffin-embedded tissues to quantificate axonal densities in the cortico-spinal tracts. In this protocol, the tissues can be distorted compared to the conventional fixation using glutaraldehyde and epon. The results can vary more than those from other histological techniques.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

INTRODUCTION

Motor neuron disease (MND) constitutes a group of heterogeneous neurodegenerative diseases that are associated with progressive upper (UMN) and / or lower motor neuron (LMN) degeneration. A portion of MND cases have genetic causes; however, the majority of MND cases are sporadic and of unknown etiology. Amyotrophic lateral sclerosis (ALS) constitutes the majority of MND cases. ALS is a clinicopathological disorder that clinically presents with progressive UMN and LMN symptoms / signs. Neuropathologically, both the UMN and LMN systems exhibit neuronal loss and gliosis, and Bunina bodies are detected in surviving neurons. Although various immunohistochemical profiles have been identified in ALS patients, 43-kDa TAR DNA-binding protein (TDP-43) is the major pathological protein in sporadic ALS.[1]

In contrast, MND that presents with LMN symptoms / signs alone occurs in several disorders, including the genetically mediated disorders spinal muscular atrophy (SMA), symmetrical axonal neuropathy, and spinal and bulbar muscular atrophy (SBMA).[2, 3] Additionally, a sporadic and adult-onset LMN disease has been referred to as progressive muscular atrophy (PMA).[3, 4] Although the revised El Escorial criteria, the standard diagnostic criteria for ALS, exclude patients who only present with LMN symptoms / signs, several studies have revealed that a subset of clinically diagnosed PMA patients exhibit the neuropathological hallmarks of ALS. Post-mortem histopathological studies have revealed cortico-spinal tract (CST) degeneration in more than half of the MND patients clinically

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

limited to LMN symptoms / signs.[5, 6] TDP-43-immunoreactive inclusions have been detected in both the LMNs and cortical neurons of PMA patients.[7, 8] The disease course of PMA is relentlessly progressive although somewhat longer than that of ALS.[2, 4, 9, 10]

However, it is unclear whether clinically diagnosed PMA is simply a clinical phenotype of ALS in which UMN symptoms / signs are undetectable. In this study, we investigated the clinicopathological profiles of clinically diagnosed PMA patients compared with those of clinically diagnosed ALS patients using a series of consecutive adult-onset sporadic MND autopsy cases.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

METHODS

Patients and clinical evaluations

We enrolled 130 consecutive autopsied patients who were clinically diagnosed with and pathologically confirmed as suffering from sporadic, adult-onset MND at the Department of Neuropathology of the Institute for Medical Science of Aging at Aichi Medical University from January 1998 to December 2010. All of the patients had been clinically evaluated by neurological experts at the Nagoya University Hospital, the Aichi Medical University Hospital, or their affiliated hospitals. Permission to perform an autopsy and archive the brain and spinal cord for research purposes was obtained from the patient's relatives by the attending physician after death. We evaluated the clinical profiles of the included patients by retrospectively reviewing case notes written both at diagnosis and in the advanced disease stage. The disease onset was defined as the time at which the patients became aware of muscle weakness. The inclusion criteria for MND patients were as follows: older than 18 years at disease onset; no family history of ALS, PMA, progressive lateral sclerosis, inherited SMA or SBMA, or any other neurodegenerative disorder; motor neuron involvement based on neurological examination; and neuropathological evidence of neuronal loss and gliosis in the UMN and / or LMN systems~~, which~~ that were not due to any cerebrovascular diseases, metabolic disorders, genetic neurological disorders, inflammatory disorders, neoplasms, or traumas. We excluded 22 patients due to invalid clinical data and 1 patient with only UMN symptoms / signs throughout his disease course, and 107 patients were

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

ultimately included in this study. Based on the clinical data, we separated these 107 MND patients into two groups, namely clinical PMA and clinical ALS groups. According to a previous study,[4] clinical PMA was defined by neurological evidence of LMN involvement (decreased or diminished deep tendon reflexes and muscle atrophy) and lack of UMN symptoms / signs (increased jaw jerk, other exaggerated tendon reflexes, Babinski sign, other pathological reflexes, forced crying, and forced laughing) throughout the clinical course. Patients who exhibited motor conduction block(s) based on extensive standardized nerve conduction studies,[11] exhibited objective sensory signs (apart from mild vibration sensory disturbances in elderly patients), or had a history of diseases that may mimic MND (e.g., spinal radiculopathy, poliomyelitis, and diabetic amyotrophy) were not included in the clinical PMA group.[4] We defined clinical ALS, based on the revised El Escorial criteria, as fulfilling 'possible' or above categories, which require UMN signs / symptoms in at least 1 region of the body.[12]

Pathological evaluations

For the pathological evaluations, we excluded one clinical PMA patient due to severe anoxic changes in the brain and 4 clinical ALS patients due to insufficient tissue material. Ultimately, we enrolled 13 clinical PMA patients for pathological evaluations. ~~and compared them with 29 clinical ALS patients who were autopsied after January 2006.~~ For comparison, we enrolled 29 clinical ALS patients who

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

were consecutively autopsied during the last 5 years of the study period (after January 2006). In addition, 13 age-matched controls (mean age at death: 68 ± 6.91 years) were enrolled. We prepared 8-mm coronal sections of the cerebrum and 5-mm axial sections of the brainstem. The tissues were fixed using 20% neutral-buffered formalin, embedded in paraffin, and sectioned at a thickness of 4.5 μm. We evaluated the sections from the precentral gyrus (4 segments from the left hemisphere), hippocampus, brainstem, and spinal cord. In all cases, the spinal cord was examined at all segment levels. Two investigators (Y. R. and M. Y.) evaluated the degeneration of the motor neuron systems and the immunohistochemical profiles of the included patients. The investigators were completely blinded to each patient's ID and the clinical diagnosis corresponding to each specimen. With respect to the degeneration of the motor neuron systems, the severity of motor neuron loss in the primary motor cortex, facial and hypoglossal nuclei, and spinal anterior horns; myelin pallor within the CST; and aggregation of macrophages within the primary motor cortex and CST were evaluated. The evaluations were performed on the most severely affected lesions and graded as none (-), mild (+), moderate (++), or severe (+++) (Fig. 1). The immunohistochemical profiles were evaluated using anti-pTDP-43 and anti-fused-in-sarcoma (FUS) antibodies in the LMN system and cerebrum. For the routine neuropathological examinations, the sections were subjected to hematoxylin-eosin (HE) or Klüver-Barrera (KB) staining. Immunohistochemistry was performed according to a standard polymer-based method using the EnVision Kit (Dako ~~Corporation~~, Glostrup, Denmark). The primary

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

antibodies used in this study were anti-ubiquitin (polyclonal rabbit, 1:2000; Dako, Glostrup, Denmark), anti-TDP-43 (polyclonal rabbit, 1:2500; ProteinTech, Chicago, IL, USA), anti-phosphorylated TDP-43 (pTDP-43 ser 409/410, polyclonal rabbit, 1:2500; CosmoBio, Tokyo, Japan), anti-FUS (polyclonal rabbit, 1:500; Sigma Aldrich, St. Louis, MO, USA), anti-alpha internexin (monoclonal mouse, 1:1000; Invitrogen, Carlsbad, CA, USA), anti-peripherin (polyclonal rabbit, 1:200; Millipore, Billerica, MA, USA), anti-CD68 (monoclonal mouse, 1:200; Dako, Glostrup, Denmark), ~~and~~ anti-phosphorylated neurofilament (pNF, monoclonal mouse, 1:600; Dako, Glostrup, Denmark), and anti-parvalbumin (polyclonal mouse, 1:1000; Sigma Aldrich, St. Louis, MO, USA). Diaminobenzidine (Wako, Osaka, Japan) was used as the chromogen.

Quantitative analysis of large axonal fibers in the CST

To evaluate the degeneration of large axonal fibers in the CST, we calculated the density of large axonal fibers in the lateral column of the spinal cord. Specimens corresponding to the C5-6 levels, immunostained using the pNF antibody, were prepared for all of the patients and 13 controls. For this assay, the paraffin-embedded spinal cords were immunostained using the anti-phosphorylated neurofilament antibody and diaminobenzidine as chromogen without additional nuclear staining to visualize only axons as brown particles. The microscopic views were binarized and automatically recognized using Luzex AP® software (Nireco, Tokyo, Japan) that was coupled to the microscope via a

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

CCD video camera. This software automatically measured the particle counts and diameters on the binarized pictures.[13] Axonal counts were evaluated on 5 areas of 10,000 μm2 (×40 objective) randomly chosen from the CST of the spinal lateral column in each patient and averaged. To validate duplicability between tests, we constructed two axon size histograms from 13 ipsilateral control samples (Supplementary File). Briefly, the variability between the test and re-test was sufficiently small to count the axons for each axon size. We constructed a histogram of axonal sizes in the CST (Fig. 3A), and the density of the large axons (axonal fibers / 10,000 μm2) was calculated (Fig. 3B-C) for the PMA and ALS patients and control samples. ~~The density (axonal fibers / 10,000 μm^2) of large pNF-positive axons that were more than 1 μm in diameter was automatically calculated using Lusex AP® software (Nireco, Japan) (Fig. 3a-b). The average values from 5 fields (×40 objective) were collected.~~

Statistical analysis

The demographic features of the PMA and ALS patients were compared using the Mann-Whitney U test for continuous variables or the Pearson chi-squared test or Fisher's exact test to assess bivariate correlations. The Kruskal-Wallis test was used for analyses between three groups, and the t t-test was used for analyses between two groups. The significance level was set at a p-value of 0.05 for comparisons between two groups and 0.016 for comparisons between three groups. All of the statistical tests performed were two-sided and were conducted using the software program PASW 18.0 (IBM®

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

SPSS®).

RESULTS

Demographic features of the registered patients

The included patients consisted of 67 men and 40 women. The mean age at disease onset was 62.7 ± 12.4 years, and the median duration from disease onset to death was 27 months (range: 2-348 months). Seventeen patients were treated with tracheostomy positive pressure ventilation (TPPV). The initial symptoms included upper-limb weakness in 40.2%, lower-limb weakness in 32.7%, bulbar symptoms in 24.3%, and respiratory symptoms in 2.8% of the included patients. Fourteen (13.1%) patients were categorized into the clinical PMA group, and 93 (86.9%) patients were classified into the clinical ALS group. With regard to clinical diagnosis, 10 (71.4%) of 14 clinical PMA patients and 88 (94.6%) of 93 clinical ALS patients were correctly diagnosed as PMA or ALS by the first referred physicians. However, 1clinical PMA patient and 4 of clinical ALS patients were initially diagnosed as having cervical or lumbar canal stenosis based on focal weakness restricted to one upper or lower limb and canal stenosis on MRI. One of the clinical PMA patients was initially diagnosed as having carpal tunnel syndrome based on weakness restricted to distal area of the median nerve in the right hand. One of clinical PMA was diagnosed as having polyradiculopathy because the cauda equina was slightly enhanced on gadolinium-enhanced MRI. One of clinical PMA patients was initially diagnosed as

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

having myositis based on myalgia and slight lymphatic infiltration on a muscle biopsy. One of clinical ALS patients was initially diagnosed as having parkinsonian syndrome because the patient showed bradykinesia due to marked rigo-spasticity in the limbs. The demographic features of the clinical PMA and ALS patients are presented in Table 1. In summary, no significant differences in the age at onset, male to female ratio, clinical duration (whether including or excluding the TPPV treatment period), or initial symptoms were detected between the clinical PMA and ALS groups.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

Table 1. Demographic features of clinical PMA and ALS patients

	Clinical PMA	Clinical ALS	p value
Number of patients	14	93	
Age at onset (years, mean ± S.D.)	60.8 ± 10.8	63.0 ± 12.7	0.388 [a]
Male / Female	10/4	57/36	0.563 [b]
Duration from onset to death (months) (median, range) [c]	21 (5-192)	29 (2-348)	0.764 [a]
Initial symptoms (number of patients)			
Bulbar symptoms	3 (21.4%)	23 (24.7%)	0.738 [b]
Upper-limb weakness	5 (35.7%)	38 (40.9%)	0.738 [b]
Lower-limb weakness	5 (35.7%)	30 (32.3%)	0.738 [b]
Respiratory symptoms	1 (7.1%)	2 (2.2%)	

[a] Mann-Whitney U test; [b] Fisher's exact test; [c] including the TPPV treatment period

Pathological evaluations

Degeneration in the UMN system (Fig. 2)

Loss of Betz cells in the primary motor cortex: Ten (76.9%) of the 13 clinical PMA patients exhibited a loss of Betz cells, which was severe in 3 (23.1%) of these patients. However, in 2 (15.4%)

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

of the 13 clinical PMA patients, no loss of Betz cells or gliosis in the primary motor cortex was detectable. In contrast, all of the patients diagnosed with clinical ALS exhibited a loss of Betz's cells, which was severe in 10 (34.5%) of the 29 clinical ALS patients. There was no significant difference in the severity of this pathological change between the clinical groups.

Aggregation of macrophages in the primary motor cortex: The ~~A~~aggregation of CD-68-positive macrophages in the primary motor cortex was detected in 10 (76.9%) of the 13 clinical PMA patients. In contrast, all of the patients diagnosed with clinical ALS exhibited aggregation of macrophages in the primary motor cortex. When comparing the clinical groups, this pathological change was significantly more severe in clinical ALS than clinical PMA ($p = 0.048$).

CST degeneration: Myelin pallor was present in 8 (61.5%) of the 13 clinical PMA patients. The ~~A~~aggregation of macrophages within the CST was detected in 11 (84.6%) of the 13 clinical PMA patients. In the clinical ALS group, all patients exhibited both myelin pallor and macrophage aggregation in the CST. When comparing the clinical groups, this pathological change was significantly more severe in clinical ALS than clinical PMA ($p = 0.004$).

Degeneration in the LMN system (Fig. 2)

All of the patients diagnosed with either clinical PMA or ALS exhibited neuronal loss in the spinal anterior horns. This neuronal loss was severe in 11 (84.6%) of the 13 clinical PMA and 20 (69.0%) of

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

the 29 clinical ALS patients. All of the patients diagnosed with clinical PMA and 27 (93.1%) of the 29 clinical ALS patients exhibited neuronal loss in the cranial nerve nuclei. This neuronal loss was severe in 6 (46.2%) of the 13 clinical PMA patients and 11 (37.9%) of the 29 clinical ALS patients. When comparing the clinical groups, there was no significant difference in the severity of LMN loss. Eight (61.5%) of the 13 clinical PMA patients and 24 (82.8%) of the 29 clinical ALS patients displayed Bunina bodies in the LMN system.

Immunohistochemical profiles (Fig. 2)

In 11 (84.6%) of the clinical PMA patients, we detected ubiquitin- and TDP-43-positive neuronal cytoplasmic inclusions (NCIs) in the LMN. In 8 of these patients, TDP-43-positive NCIs were also detected in the primary motor cortex. All of the clinical ALS patients displayed ubiquitin- and TDP-43-positive NCIs in the LMN system. Moreover, TDP-43-positive glial cytoplasmic inclusions were observed in the spinal anterior horn and primary motor cortex in all of the TDP-43-positive patients of the clinical ALS and PMA groups. In contrast, 2 of the clinical PMA patients (15.4%) exhibited basophilic inclusion bodies in the neuronal cytoplasm, which were broadly extended throughout the central nervous system. These inclusions were positive for FUS but negative for TDP-43, alpha-internexin, and peripherin. ~~All of the clinical ALS patients displayed ubiquitin- and TDP-43-positive NCIs in the LMN system.~~

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

Quantitative analysis of large axonal fibers in the CST (Fig. 3)

~~The average density of the large axonal fibers in the CST was as follows:~~The histogram of axonal sizes revealed that the percentage of axons that were greater than 1 μm in diameter were smaller in ALS (18.5%) and PMA (23.9%) than in controls (32.3%), resulting in a relative increase of the percentage of smaller axons. Then, we measured the densities of large axons densities that were greater than 1 μm in diameter. The average densities were as follows: –clinical ALS, 68.3 ± 20.9 fibers / 10,000 μm^2; clinical PMA, 97.2 ± 31.5 fibers / 10,000 μm^2; and controls, 129.1 ± 6.1 fibers / 10,000 μm^2 ($p = 0.001$ between the clinical ALS and PMA groups; $p = 0.001$ between the clinical PMA and control groups; $p < 0.001$ between the clinical ALS and control groups). All of the patients diagnosed with clinical ALS exhibited lower values than the range of normal values that was obtained from the controls. In contrast, the results from the clinical PMA group were widely diverse. The results from 5 (38.5%) of the 13 clinical PMA patients were within the normal range, but 8 (61.5%) of these patients exhibited lower values than the normal range. One PMA patient who had been treated with TPPV exhibited an exceptionally low value.

Pathological overview of the patients diagnosed with clinical PMA or clinical ALS

Clinical PMA: Eleven (84.6%) of the 13 clinical PMA patients displayed both UMN degeneration

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

(either the loss of Betz cells, myelin pallor, or the aggregation of macrophages in the primary cortex or CST) and LMN degeneration. Nine of these patients exhibited TDP-43-positive inclusions, and the remaining 2 patients displayed FUS-positive basophilic inclusion bodies. Their large CST axon densities were diverse, ranging from low values to values within the normal range that was obtained from the control subjects. In 2 (15.4%) of the 13 clinical PMA patients, neuropathological parameters that we defined as the UMN system degeneration were all negative. ~~degeneration in the UMN system was undetectable.~~ Their large CST axon density was within the normal range. These 2 patients exhibited abundant TDP-43-positive neuronal and glial inclusions in the LMN and, occasionally, in layers II-III of the primary motor cortex and the hippocampus. The pathological findings from the representative patients are shown in Fig. 4. ~~Their CST axon density was within the normal range.~~

Clinical ALS: All 29 patients displayed a combination of UMN and LMN system degeneration and exhibited TDP-43-positive inclusions.

Additionally, of the respirator-managed patients, 3 patients (Patient 13 of clinical PMA and Patients 27 and 28 of clinical ALS) showed diffusely extended neuronal loss, gliosis, and TDP-43 pathology beyond the motor neuron systems, which involved all layers of the cerebral neocortices, the striatum, the thalamus, the cerebellar dentate nucleus, and the non-motor nuclei in the brainstem, including the substantia nigra, the red nucleus, the periaqueductal gray matter, the inferior olivary nucleus, and the reticular formation.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

~~**Case presentation**~~

~~Patient 2 in the clinical PMA group in Fig. 2~~

~~This patient was a woman who died at 75 years of age. At the age of 73 years, she presented with weakness in her left hand. A neurological examination revealed atrophy of the abductor pollicis brevis muscle in the left hand. The tendon reflexes were generally reduced, and there were no pathological reflexes. At the age of 74 years, a neurological examination revealed diffuse weakness and muscular atrophy in her upper limbs. Electromyographic analysis revealed active denervation in all four limbs and the tongue. A diagnosis of PMA was made. She died of respiratory failure 1.5 years after disease onset. No clinical UMN signs or symptoms were observed throughout her disease course. Based on a postmortem study, degeneration of the UMN system was undetectable (Fig. 4a-e), although TDP-43-positive neuronal and glial inclusions were occasionally detected in layers II-III of the motor cortex, the hippocampal dentate gyrus, and the parahippocampal gyrus (Fig. 4f, g). Severe neuronal loss, TDP-43-positive NCIs, and Bunina bodies in the LMN system were detected (Fig. 4h-j).~~

~~Patient 12 in the clinical PMA group in Fig. 2~~

~~This patient was a woman who died at 76 years of age. At the age of 74 years, she presented with weakness in her lower limbs. A neurological examination revealed muscular atrophy in her tongue,~~

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

lower limbs, and hands. The tendon reflexes were reduced in her limbs, and there were no pathological reflexes. Electromyographic analysis revealed active denervation in the left quadriceps femoris muscle. She died of respiratory failure 2.0 years after disease onset. No clinical UMN signs or symptoms were observed throughout her disease course. A postmortem study revealed marked degeneration in both the UMN and LMN systems (Fig. 4k-m). TDP-43-positive neuronal and glial inclusions were detected in the spinal anterior horn, cranial nerve nuclei, motor cortex, hippocampal dentate gyrus, and parahippocampal gyrus.

Patient 6 in the clinical PMA group in Fig. 2

This patient was a man who died at 62 years of age. At the age of 60 years, he presented with weakness in the upper limbs. A neurological examination revealed muscular atrophy of the shoulder girdles and arms. The tendon reflexes were generally absent, and there were no pathological reflexes. Three months following diagnosis, weakness in the respiratory muscles occurred, which was followed by management with TPPV. He died of pneumonia 1.5 years after disease onset. No clinical UMN signs or symptoms were observed throughout his disease course. A postmortem study revealed severe neuronal loss in the spinal anterior horn and mild neuronal loss in the primary motor cortex. Abundant FUS-positive basophilic inclusion bodies were detected in the neurons of the spinal anterior horn, brainstem, basal ganglia, and cerebral cortex (Fig. 4n, o). We performed genetic analysis of the FUS

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

gene using frozen brain tissue with the consent of family members. Direct sequencing revealed no mutations in any of the 15 exons or the exon/intron boundary sequences of the FUS gene in this patient.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

DISCUSSION

Our study demonstrated the clinicopathological profiles of clinical PMA and ALS patients in a consecutive autopsy series. The clinical evaluations in this study revealed rapid disease progression and short survival duration in clinical PMA patients, which are analogous courses ~~analogous~~ to those that are characteristic of clinical ALS. Contrary to our results, it has been described that PMA exhibits slower progression and longer survival duration compared with ALS.[3] However, recent studies revealed that PMA follows a relentlessly progressive course and that the survival duration is not much longer than that of ALS.[2, 4, 9, 10, 143] The relatively small number of patients in our study may have contributed to the absence of significant differences in the survival durations between the clinical PMA and ALS groups.

Our pathological results indicate that, ~~clinical PMA consists of three patterns of neuropathological features: UMN and LMN degeneration with TDP-43 pathology, consistent with ALS; LMN degeneration with TDP-43 pathology but no UMN degeneration; and UMN and LMN degeneration with FUS-positive basophilic inclusion bodies. O~~ of the clinical PMA patients, 85% exhibited degeneration in both the UMN and LMN systems, ~~corresponding~~ which corresponds to ALS. However, the remaining 15% of the clinical PMA patients lacked any apparent degeneration in the UMN system. A previous study reported that approximately 50% of all PMA patients exhibit ~~the presence of~~ macrophages in the CST.[5] Another report demonstrated th~~at~~ degeneration of the

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

pyramidal tract and loss of Betz cells ~~were found~~ in 65% and 60%, respectively, of the patients diagnosed with the PMA phenotype.[6] Our results revealed that PMA patients more frequently had degeneration in the UMN system than those reported in previous studies; however, in a few PMA patients, the UMN degeneration remained undetectable at death. Our pathological results revealed differential UMN involvement between PMA patients and indicated that PMA and ALS are in a continuous pathological entity. Regarding immunohistochemical aspects, several studies have revealed that TDP-43 pathology is commonly observed in the cerebral cortices or the subcortical gray matters of PMA patients.[7,8] In our results, TDP-43-positive neuronal or glial inclusions in the motor cortices or hippocampus was common both in clinical ALS and PMA groups and was found even in patients apparently lacking UMN degenerative changes. A recent report described the propagation of TDP-43 pathology in ALS, which starts from the UMN and LMN systems and spreads to the antero-medial temporal lobes through the motor neuron system.[15] Based on this theory of TDP-43 propagation, TDP-43 pathology beyond the LMN system in PMA patients may support the pathological continuity between these two clinical phenotypes. ~~However, in a few PMA patients, UMN degeneration remained undetectable at death.~~

~~One limitation of our study was the inability to evaluate the entire motor cortex and CST. It is controversial whether patients with no apparent degeneration of the UMN system have ALS with extremely mild UMN involvement or another pathology that is confined to the LMN system. However,~~

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

For peer review only

~~the patients who lacked any apparent UMN degeneration displayed TDP-43-positive NCIs in the primary motor cortex and the hippocampal dentate gyrus, although these inclusions were limited to a very low level. A recent report described the propagation of TDP-43 pathology in ALS, which starts from the UMN and LMN systems and spreads to the antero-medial temporal lobes through the motor neuron system.[14] Based on this theory of TDP-43 propagation, the 2 patients who apparently lacked UMN degeneration could be included in the pathological spectrum of ALS.~~

The standard diagnostic criteria for ALS are the revised El Escorial criteria, which require a combination of UMN and LMN symptoms / signs for the diagnosis of ALS.[12] However, it is often difficult to clinically determine whether the UMN is involved,[16] which sometimes results in diagnostic difficulty. In our patient series, only 71.4% of the clinical PMA patients were correctly diagnosed by the first referred physicians, although 94.6% of the clinical ALS patients were.~~However,~~ ~~it is often difficult to clinically determine whether the UMN is involved, as UMN signs can be masked by severe coexisting LMN symptoms or signs.[15]~~ Recently, several studies have demonstrated the utility of radiological procedures, including transcranial magnetic stimulation, ^{1}H magnetic resonance spectroscopy, and diffusion tensor imaging, in the detection of UMN system deterioration in a subset of PMA patients.[14~~3~~, 17~~5~~-20~~18~~] Based on our results, a large subset of PMA patients may have some degree of UMN degeneration. In such patients, these radiological or electrophysiological procedures would be expected to increase the sensitivity of detection of UMN degeneration. However, our results

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

also indicate that some of the PMA patients exhibit sparse morphological changes in the UMN system, even at death. ~~In such patients, i~~It may be difficult to detect UMN degeneration ~~even~~ using these procedures in such patients. To diagnose clinical PMA patients displaying sparse UMN degeneration as ALS in the early phase of the disease course may be a future subject of focus.

A limitation of our study was the inability to evaluate the entire motor cortex and CST, and it is controversial whether patients apparently intact UMN systems actually lack or have extremely mild UMN involvement. Another methodological limitation is that we evaluated axonal sizes and densities using neutral formalin-fixed, paraffin embedded specimens. The tissues may be somewhat distorted when compared with conventional nerve fixation using glutaraldehyde followed by epon embedding. Our methods were considered to be appropriate to assess the proportional changes in sizes of pyramidal axons, but the absolute values of axonal diameters can vary from those that have have been obtained using other histological techniques.[13]

In summary, 84.6% of clinical PMA patients displayed both UMN and LMN degeneration, which is consistent with the pathological profiles of ALS. In 15.4% of the clinical PMA patients, degeneration in the UMN system was undetectable. The large axon density in the CST varied from low values to a normal range. In contrast, all of the clinical ALS patients displayed a combination of UMN and LMN system degeneration and significantly reduced large axon density in the CST.

~~Immunohistochemically, our clinical PMA patients exhibited TDP-43 pathology or~~

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

For peer review only

~~FUS-positive BIBD. TDP-43 is considered to be the major aggregated protein in PMA and ALS. In contrast, BIBD is known as a rapidly progressive, sporadic or familial MND with a younger age of onset.[19-22] However, patients with a higher age of onset have been occasionally described, such as Patient 6 in the clinical PMA group.[23-25]~~

~~In conclusion, the neuropathological profiles of clinical PMA consisted of three patterns: UMN and LMN degeneration with TDP-43 pathology, consistent with the pathological profile of ALS; LMN degeneration with TDP-43 pathology but sparing of the UMN system; and UMN and LMN degeneration with FUS-positive BIBD. One subject of future focus is how to diagnose PMA patients with few UMN degenerations as ALS in the early disease phase.~~

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

ACKNOWLEDGEMENTS

Contributors YR and NA contributed to the conception and design of the study. All of the authors participated in the acquisition, analysis, and interpretation of the data. MY and GS drafted the manuscript. IM and WH assisted in writing and editing the manuscript.

Funding This work was supported by Grants-in-Aid from the Research Committee of CNS Degenerative Diseases of the Ministry of Health, Labor, and Welfare of Japan.

Conflicts of interest The authors declare that they have no conflicts of interest.

Ethics approval This study was approved by the ethics committees of Nagoya University and Aichi Medical University.

We give special thanks to Dr. M. Hasegawa, Department of Neuropathology and Cell Biology, Tokyo Metropolitan Institute of Medical Science, for performing the genetic analysis of the FUS genes.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

REFERENCES

1. Neumann M, Sampathu DM, Kwong LK, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 2006;314:130–3.

2. de Carvalho M, Scotto M, Swash M, et al. Clinical patterns in progressive muscular atrophy (PMA): a prospective study. Amyotroph Lateral Scler 2007;8:296-9.

3. Norris FH. Adult progressive muscular atrophy and hereditary spinal muscular atrophies. In: Vinken PJ, Bruyn GW, Klawans HL, De Jong JMBV eds. Handbook of Clinical Neurology: Diseases of the Motor System, vol. 59. Amsterdam: North-Holland Publishing Company 1991:13-34.

4. Visser J, van den Berg-Vos RM, Franssen H, et al. Disease course and prognostic factors of progressive muscular atrophy. Arch Neurol 2007;64:522-8.

5. Ince PG, Evans J, Knopp M, et al. Corticospinal tract degeneration in the progressive muscular atrophy variant of ALS. Neurology 2003;60:1252-8.

6. Brownell B, Oppenheimer DR, Hughes JT. The central nervous system in motor neurone disease. J

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

Neurol Neurosurg Psychiat 1970;33:338-57.

7. Geser F, Stein B, Partain M, et al. Motor neuron disease clinically limited to the lower motor neuron is a diffuse TDP-43 proteinopathy. Acta Neuropathol 2011;121:509-17.

8. 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.

9. Kim WK, Liu X, Sandner J, et al. Study of 962 patients indicates progressive muscular atrophy is a form of ALS. Neurology 2009;73:1686-92.

10. Van den Berg-Vos RM, Visser M, Kalmijn S, et al. A long-term prospective study of the natural course of sporadic adult-onset lower motor neuron syndromes. Arch Neurol 2009;66:751-7.

11. Koike H, Hirayama M, Yamamoto M, et al. Age associated axonal features in HNPP with 17p11.2 deletion in Japan. J Neurol Neurosurg Psychiat 2005;76:1109-14.

12. Brooks BR, Miller RG, Swash M, et al. El Escorial revisited: revised criteria for the diagnosis of

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord 2000;1:293-9.

13. Sobue G, Hashizume Y, Mitsuma T, et al. Size-dependent myelinated fiber loss in the corticospinal tract in Shy-Drager syndrome and amyotrophic lateral sclerosis. Neurology 1987;37:529-32.

143. Mitsumoto H, Ulug AM, Pullman SL, et al. Quantitative objective markers for upper and lower motor neuron dysfunction in ALS. Neurology 2007;68:1402-10.

154. Brettschneider J, Del Tredici K, Toledo JB, et al. Stages of pTDP-43 pathology in amyotrophic lateral sclerosis. Ann Neurol 2013;74:20-38.

16. Swash M. Why are upper motor neuron signs difficult to elicit in amyotrophic lateral sclerosis? J Neurol Neurosurg Psychiat 2012;83:659-62.

175. Sach M, Winkler G, Glauche V, et al. Diffusion tensor MRI of early upper motor neuron involvement in amyotrophic lateral sclerosis. Brain 2004;127:340-50.

18. Vucic S, Ziemann U, Eisen A, et al. Transcranial magnetic stimulation and amyotrophic lateral

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

sclerosis: pathophysiological insights. J Neurol Neurosurg Psychiatry 2013;84:1161-70.

~~16. Floyd AG, Yu QP, Piboolnurak P, et al. Transcranial magnetic stimulation in ALS: utility of central motor conduction tests. Neurology 2009;72:498-504.~~

197. Graham JM, Papadakis N, Evans J, et al. Diffusion tensor imaging for the assessment of upper motor neuron integrity in ALS. Neurology 2004;63:2111–9.

2018. Prudlo J, Bißbort C, Glass A, et al. White matter pathology in ALS and lower motor neuron ALS variants: a diffusion tensor imaging study using tract-based spatial statistics. J Neurol 2012;259:1848–59.

~~19. Munoz DG, Neumann M, Kusaka H, et al. FUS pathology in basophilic inclusion body disease. Acta Neuropathol 2009;118:617–27~~

~~20. Neumann M, Roeber S, Kretzschmar HA, et al. Abundant FUS-immunoreactive pathology in neuronal intermediate filament inclusion disease. Acta Neuropathol 2009;118:605–16.~~

~~21. Mackenzie IRA, Ansorge O, Strong M, et al. Pathological heterogeneity in amyotrophic lateral~~

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

sclerosis with FUS mutations: two distinct patterns correlating with disease severity and mutation. Acta Neuropathol 2011;122:87–98.

22. Yokota O, Tsuchiya K, Terada S, et al. Basophilic inclusion body disease and neuronal intermediate filament inclusion disease: a comparative clinicopathological study. Acta Neuropathol 2008;115:561–575.

23. Matsuoka T, Fujii N, Kondo A, et al. An autopsied case of sporadic adult-onset amyotrophic lateral sclerosis with FUS-positive basophilic inclusions. Neuropathology 2011;31:71–6.

24. Sasaki S, Toi S, Shirata A, et al. Immunohistochemical and ultrastructural study of basophilic inclusions in adult-onset motor neuron disease. Acta Neuropathol 2001;102:200–6.

25. Ishihara K, Araki S, Ihori N, et al. An autopsy case of frontotemporal dementia with severe dysarthria and motor neuron disease showing numerous basophilic inclusions. Neuropathology 2006;26:447–454.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

FIGURE LEGENDS

Figure 1 Measures of degeneration in the upper motor neuron system. (A-D) Loss of Betz cells in the primary motor cortex: stage (-), the Betz cells were spared in number, and gliosis was absent (A); stage (+), mild neuronophagia and gliosis were noted (B); and stage (++), marked neuronophagia and glial proliferation were observed (C). (D-K) Aggregation of CD-68-positive macrophages in the primary motor cortex (D-G) and the corticospinal tract in the lateral column of the spinal cord (H-K): stage (-), the aggregates were absent (D, H); stage (+), the aggregates were occasionally present (E, I); stage (++), the aggregates were present at a number of 1-5 / ×100 field (F, J); and stage (+++), the aggregates were diffusely observed (G, K). L-O Myelin pallor in the CST of the lateral column of the spinal cord: stage (-), myelin pallor was not detected (L); stage (+), myelin pallor was slightly notable (M); stage (++), myelin pallor was moderate (N); and stage (+++), the CST was entirely pale. (A-C) hematoxylin-eosin staining, (D-K) anti-CD-68 immunohistochemistry, and (L-O) Klüver-Barrera staining. Scale bars: (A-G) 100 μm, (H-K) 50 μm, and (L-O) 3 mm.

Figure 2 Summary of the neuropathological findings in the included patients. The stages of the pathological changes correspond to Figure 1. Pathological changes between the clinical groups were compared using Pearson's chi-squared test. Abbreviations: GCI, glial cytoplasmic inclusions; KB, Klüver-Barrera staining; NCI, neuronal cytoplasmic inclusion; TPPV, tracheostomy positive pressure

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

ventilation.

Figure 3 Quantitative analysis of the axonal fibers in the corticospinal tract. (A) Phosphorylated neurofilament (pNF)-positive fibers were automatically binarized using Luzex AP® software. The density of pNF-positive axons (particles / 10,000 μm 2) was automatically calculated using averaged data from 5 fields (×400). The histogram of axonal sizes revealed that the percentages of axons that were more than 1 μm in diameter were smaller in ALS and PMA than in controls. (B) The large axonal fibers more than 1 μm in diameter were automatically recognized, binarized, and counted using the software to successfully evaluate the axonal density.~~Quantitative analysis of the large axonal fibers in the corticospinal tract. (A, B) To successfully evaluate the density of the large axonal fibers, phosphorylated neurofilament (pNF)-positive fibers that were more than 1 μm in diameter were automatically imaged using Lusex AP® software. The density (axonal fibers / 10,000 μm^2) of the large pNF-positive axons was automatically calculated using averaged data from 5 fields (×400).~~ (C) There were significant differences between all pairs of clinical groups: $p = 0.001$ (*) between the clinical ALS and clinical PMA groups, $p = 0.001$ (*) between the clinical PMA and control groups, and $p < 0.001$ (**) between the clinical ALS and control groups. All patients diagnosed with clinical ALS exhibited lower values than the controls. In contrast, the results of the clinical PMA group were widely diverse, ranging from low values to values within the normal range.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.

Figure 4 Neuropathological profiles of the patients in the clinical PMA group. A-J correspond to Patient 2. The CST did not display myelin pallor (A), loss of large axonal fibers (B), or aggregation of macrophages (C). Additionally, in the primary motor cortex, neither loss of Betz cells (D) nor aggregation of macrophages (E) was detected. The upper layers of the primary motor cortex rarely contained phosphorylated 43-kDa TAR DNA-binding protein (pTDP-43)-positive neuronal (F) and glial (G) inclusions. The spinal anterior horn displayed severe neuronal loss (H), pTDP-43-positive skein-like inclusions (I), and Bunina bodies (J). K-M correspond to Patient 12. The CST displayed myelin pallor (K) and depletion of large axonal fibers (L). Neuronophagia was often found in the primary motor cortex (M, arrows). (N-O) correspond to Patient 6. The spinal motor neurons contained basophilic inclusion bodies (N) that were positive for anti-fused-in-sarcoma (FUS) based on immunohistochemistry (O). (A, K) Klüver-Barrera staining, (B) anti-phosphorylated neurofilament immunohistochemistry, (C, E) anti-CD-68 immunohistochemistry, (D, H, J, M) Hematoxylin-eosin staining, (F, G, I) anti-pTDP-43 immunohistochemistry, and (O) anti-FUS immunohistochemistry. Scale bars: (A, K) 3 mm, (D-E) 100 μm, (C, H, M) 50 μm, (B) 20 μm, and (F, G, I, J, N, O) 10 μm.

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2014-005213 on 14 May 2014. Downloaded from http://bmjopen.bmj.com/ on April 20, 2024 by guest. Protected by copyright.