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Familial ALS with *FUS* P525L mutation: two Japanese sisters with multiple systems involvement

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ABSTRACT

We evaluated the clinicopathological features of familial amyotrophic lateral sclerosis (ALS) with the *fused in sarcoma (FUS)* P525L mutation. Two sisters and their mother had a similar clinical course, which was characterized by the development of limb weakness at a young age with rapid disease progression. An elder sister, patient 1, progressed into a totally locked-in state requiring mechanical ventilation and died 26 years after the onset of the disease. In contrast, the younger sister, patient 2, died in the early stages of the disease. The patients had neuropathological findings that indicated a very active degeneration of motor neurons and multiple system degeneration, which led to marked brain and spinal cord atrophy in the long term clinical outcome. The multiple system degeneration included the frontal lobe, the basal ganglia and substantia nigra, cerebellum and related area. Compared with previously reported ALS cases, the severe degeneration of the frontal lobe and the striatum were the characteristic features in the patient 1 in this case study. The degeneration spread over multiple systems might be caused not only by the appearance of the FUS immunoreactive neuronal cytoplasmic inclusions but also by the degeneration of neuronal connections from the primary motor cortex and related areas.

1. Introduction

Sporadic amyotrophic lateral sclerosis (ALS) with basophilic cytoplasmic inclusions (BIs), characterized by an onset before 25 years of age with rapid progression, has been proposed to constitute a distinct clinical entity [1,2]. Bäumer et al. [3] demonstrated that the BIs in the cases of sporadic juvenile ALS were immunoreactive for fused in sarcoma (FUS), and two of their six patients had the *FUS* P525L mutation. Therefore, they proposed that juvenile ALS with BIs should be classified as ALS–FUS. Neuropathologically, Mackenzie et al. [4] divided patients with ALS–FUS in two groups: early-onset cases, including two with the *FUS* P525L mutation [3] and late-onset cases, including two with the *FUS* R521C mutation. Furthermore, they [4] suggested that patients with ALS–FUS and frontotemporal lobar degeneration (FTLD) with FUS-immunoreactive pathology (FTLD–FUS) were distinct because none of their ALS–FUS cases showed involvement of the broad range of neuroanatomical regions that occurs in the FTLD cases [5]. The neuropathological features of ALS with the *FUS* P525L mutation have been reported in only one other patient [6], who showed similar neuropathological features as to early-onset ALS–FUS [4]. This patient used non-invasive positive pressure ventilation during the last 4 months of her life; however, the other reported patients with the *FUS* P525L mutation [3,4] did not use respiratory assistance. Therefore, although it has been shown that an ALS case with the *FUS* R521C mutation, late-onset ALS–FUS showed no cerebral cortical involvement even in the prolonged stage with using mechanical ventilation [7], much is unknown about the clinicopathology of ALS with the *FUS* P525L mutation, early-onset ALS-FUS. Herein, we report the clinical and neuropathological findings in two autopsied sisters and their mother with Japanese familial ALS associated with the *FUS* P525L mutation. The two autopsied patients used mechanical ventilators: the elder sister used it for over 20 years; however, the younger sister only used it for approximately one year. Therefore, the difference between the clinicopathological

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findings of the elder sister at the late stage of the disease and those of the younger sister at the early stage of the disease are most likely indicative of the mechanism of disease progression. The significance of their neuroanatomical regions is discussed.

2. Case reports

2.1. Patient 1

The elder sister (proband, Subject III-1 in Fig. 1) exhibited a slight developmental delay in childhood. At the age of 13 years, she developed left and then right leg weakness, followed by quadriparesis. Her clinical feature was suggested to be a polyneuropathy. However, plasmapheresis was not effective for her. One year and 3 months after the onset, she was admitted to the Tokyo Metropolitan Neurological hospital because dysphagia, dyspnea, and dysarthria appeared. She was diagnosed as ALS and underwent a tracheostomy, with subsequent prolonged mechanical ventilation. Three years after the onset, she developed adduction paresis of the right eye, followed by ophthalmoparesis of both eyes. However, she was able to comprehend her situation. Head CT showed progressive brain atrophy (Fig. 2). Finally, all of her voluntary movement disappeared at the age of 23 years. Specifically, she progressed to a totally locked-in state that has been proposed [8] as one of the subgroups in the terminal condition of respirator-assisted long-survival ALS [9]. She died of pneumonia at the age of 40 years, 26 years after the onset.

2.2. Genetic analysis

In patient 1, DNA was extracted from the patient's leukocytes using a conventional method with informed consent. All of the coding regions and exon–intron boundaries of the *FUS* gene were examined by direct sequencing of polymerase chain reaction (PCR) products. Detailed information regarding the PCR amplification conditions is available from the authors upon request. Sequencing of the PCR products was performed using a BigDye Terminator Cycle Sequencing Reaction kit (Life Technologies Japan) and an ABI PRISM 3100 Genetic Analyzer (Life Technologies Japan).

The sequence analysis of the *FUS* gene identified a proline 525 to leucine (P525L) mutation. Therefore, we performed a deep resequencing analysis of the target gene and confirmed the presence of a rare heterozygous C-to-T transition at cDNA position 1574, resulting in a P525L missense mutation within the arginine-glycine-glycine motif of exon 15.

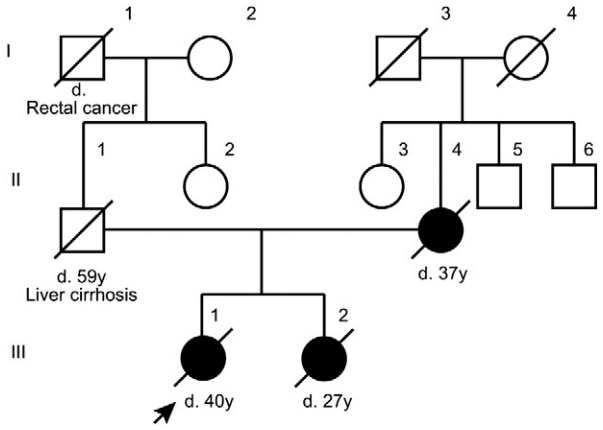

Fig. 1. Pedigree of the family. The arrow indicates the proband. The affected individuals are represented by the solid black symbols. I-4 lived to be more than 81 years old.

2.3. Patient 2

Patient 2 (Subject III-2 in Fig. 1) was the younger sister of patient 1. She developed right hand weakness at the age of 25 years. One year after, she showed gait disturbance, dysphagia, and respiratory failure. Subsequently, she was placed on mechanical ventilation and was transferred to the Mihara Memorial hospital and evaluated by a neurologist. Neurological examination at the age of 26 years showed quadriplegia with hypotonia and decreased tendon reflexes in the all extremities, while Babinski's sign appeared. She died of pneumonia at the age of 27 years.

2.4. Patient 3

The mother of patients 1 and 2 (Subject II-4 in Fig. 1) had developed left arm weakness and died of progressive bulbar palsy within 6 months of the disease duration at the age of 35 years.

3. Methods

3.1. Neuropathological study

The brain and spinal cord specimens were fixed with 20% buffered formalin and embedded in paraffin. Neuronal loss and/or fiber loss and gliosis was assessed in various regions of the nervous system using 10-μm-thick sections with hematoxylin and eosin (HE) and Klüver–Barrera (KB) stains. When necessary, Bodian, Nissl, periodic acid-Schiff (PAS) stain, luxol fast blue (LFB), cresyl violet, and Gallyas–Braak staining was performed.

3.2. Immunohistochemistry for BIs

For the immunohistochemistry, 6-μm-thick sections were prepared. Specimens of the frontal lobe, hippocampus with medial temporal lobe, and pons were immunostained for ubiquitin (DAKO, 1:600), 43-kDa TAR DNA binding protein-43 (TDP-43) (Polyclonal Protein Tech Group, 1:500), α-internexin (Cosmo Bio, 1:250), α-synuclein (Santa Cruz Biotechnology, 1:400), and phosphorylation-dependent τ (AT8; Innogenetics, 1:5000), and the specimens of each cerebral lobe, basal ganglia, cerebellum, brainstem, and spinal cord were immunostained for FUS (Sigma, 1: 100) using a labeled streptavidin–biotin method.

3.3. Electron microscopical study of BIs

Several pieces of formalin-fixed inferior frontal cortex and pontine nuclei of patient 1 were postfixed with 4% osmium tetroxide and conventionally processed for electron microscopy (Hitachi H-9000).

4. Results

4.1. Neuropathological findings in patient 1

The brain weighed 715 g. Marked cerebral atrophy was observed in the brainstem and cerebellar regions (Fig. 3A). The frontal white matter was marked with atrophy, the caudate nucleus was thin, and the putamen and globus pallidus were atrophic and brownish in color (Fig. 3B). The ventral lateral nucleus of the thalamus showed severe atrophy. However, the limbic system, including the medial temporal area, mammillary body, and cingulate gyrus were preserved.

The brainstem and spinal cord were markedly atrophic (Fig. 4A), and the anterior horn of the spinal cord (Fig. 4B) and all motor nuclei of the brainstem showed severe neuron loss and gliosis (Table 1). Although some neurons were observed in the intermediolateral nucleus, neurons in Clarke's nucleus were markedly decreased. The dorsal root ganglion cells were preserved. In the spinal cord, although the posterior column was preserved, almost all of the fibers from the other areas were lost