Title:
FALS with Gly72Ser mutation in SOD1 gene: report of a family including the first autopsy case

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ABSTRACT

Clinical information on familial amyotrophic lateral sclerosis (FALS) with Gly72Ser mutation in the Cu/Zn superoxide dismutase-1 (SOD1) gene has been limited and autosy findings remain to be clarified. We describe one Japanese family with ALS carrying Gly72Ser mutation in the SOD1 gene, in which autopsy was performed on one affected member. The autopsied female patient developed muscle weakness of the left thigh at age 66 and showed transient upper motor neuron signs. She died of respiratory failure 13 months after onset without artificial respiratory support. There were no symptoms suggesting bladder or rectal dysfunction throughout the clinical course. Her brother with ALS was shown to have Gly72Ser mutation in the SOD1 gene. Histopathologically, motor neurons were markedly decreased throughout the whole spinal cord, whereas corticospinal tract involvement was very mild and was demonstrated only by CD68 immunohistochemistry. Degeneration was evident in the posterior funiculus, Clarke's nucleus, posterior cerebellar tract, and Onuf's nucleus. Neuronal hyaline inclusions were rarely observed in the neurons of the spinal cord anterior horn including Onuf's nucleus, and were immunoreactive for SOD1. To date, neuron loss in Onuf's nucleus has hardly been seen in ALS, except in the patients showing prolonged disease duration with artificial respiratory support. Involvement of Onuf's nucleus may be a characteristic pathological feature in FALS with Gly72Ser mutation in the SOD1 gene.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder that mainly involves the upper and lower motor neurons. Familial cases account for 5-10% of all cases of ALS, and mutations of the Cu/Zn superoxide dismutase-1 (SOD1) gene, which encodes an antioxidant

enzyme, are present in about 20% of FALS patients and in about 2% of sporadic ALS (SALS) patients [1]. Over 150 mutations in the SOD1 gene have been identified in FALS and SALS cases since 1993 [2]. Within the cell, SOD1 is physiologically distributed in the cytosol, lysosomes, microsomes, mitochondria, and nuclei [3]. Misfolding and aggregation of SOD1 is related to gain of toxic function in ALS with SOD1 mutation [4]. Increasing evidence indicates that non-neuronal-neighboring cell types contribute to the pathogenesis and disease progression [5].

Clinical features of ALS with SOD1 mutation are largely indistinguishable from those of SALS; however, lower limb-onset and lower motor neuron-predominant involvement are relatively common [6]. The onset age and disease duration are variable among the mutations. Histopathologically, most cases of ALS with SOD1 mutation show Lewy body-like hyaline inclusions, which are immunoreactive for SOD1 [7-9]. The posterior funiculus, Clarke's nucleus and posterior spinocerebellar tract were also involved. The corticospinal tract (CST) involvement is slight or mild [7]. In patients with the I113T mutation, neurofilament pathology is an almost universal feature [7].

Onuf's nucleus is a small group of cells, first described by a neuroanatomist called Onufrowicz. In 1899, he found that this group of cells was localized exclusively in the second sacral segment [10]. Onuf's nucleus is functionally related to the rectum and bladder, including the external sphincter muscles of the anus and urethra [10]. Onuf's nucleus is usually preserved in ALS [10, 11], and neuron loss has hardly been seen except in the patients showing prolonged disease duration with artificial respiratory support [12-15]. Here, we report one Japanese family with ALS showing Gly72Ser mutation including the first autopsy patient, in whom both neuron loss and neuronal cytoplasmic inclusions were demonstrated in Onuf's nucleus.

2. Clinical assessment

Case II-2

The family tree is shown in Figure 1. A 75-year-old Japanese man developed muscle weakness of the fingers of the bilateral upper limbs. There had been paresis of the right upper limb since brain infarction occurred at age 65. Two patients with ALS in his family had already died (Fig. 1). Six months after onset, muscle weakness emerged at the proximal parts of the bilateral upper limbs, and nine months after onset, he became unable to produce vocal sounds. He was admitted to the Department of Neurology in the general hospital ten months after onset. On admission, there was marked tongue atrophy and fasciculation, and prominent dysarthria. Neck weakness corresponded to grade 3 in the manual muscle testing. Symmetrical muscle weakness of the bilateral upper limbs ranged from grade 3 to 4 on manual muscle testing. There was no muscle weakness in the lower limbs. Deep tendon reflex was increased in the right upper limb and bilateral lower limbs. Plantar reflexes were not elicited. There was no sensory disturbance. Needle electromyography detected active and chronic denervation potentials, and a diagnosis of familial ALS was made. DNA was extracted from his blood sample, and direct sequencing of SOD1 gene exon 3 showed missense mutation substituting glycine for serine (Gly72Ser) (Fig. 2). He died of respiratory failure ten months after onset. Autopsy could not be performed.

Case II-4

A 66-year-old woman developed muscle weakness of the left thigh, and experienced falls. Two months after onset, fasciculation emerged in the muscles of the left thigh. Four months after onset, she needed a cane while walking, then became unable to walk and was admitted to a general

hospital five months after onset. On admission, there were muscle fasciculations in the bilateral thigh and hip, and muscle atrophy and weakness in the left lower limb. Muscle weakness ranged from grade 0 to 2 on manual muscle testing. Achilles tendon reflex was absent on the left side, and was within normal limits on the right. The patellar tendon reflex was decreased on the left side and increased on the right. The upper limbs tendon reflex was bilaterally increased. There was Babinski's sign bilaterally. Vibration sensation was slightly decreased in the bilateral lower limbs. On nerve conduction study, there was no conduction block. Needle electromyography showed denervation potentials in the bilateral lower limbs and right upper limb. A diagnosis of ALS was made. At that time, information on the family histoly of ALS could not be obtained. Thereafter, muscle weakness emerged in the right lower limb.

Seven months after onset, neurological examination demonstrated flaccid paraparesis without hyperreflexia or plantar reflexes. Nerve conduction study demonstrated reduction of the amplitude of the compound muscle action potentials (CMAPs), but conduction velocity was within normal limits. Cerebrospinal fluid examination showed slight elevation of the total protein level (53 mg/dl). Because we could not completely exclude the possibility of chronic inflammatory demyelinating polyradiculoneuropathy (CIDP), prednisolone of 60mg was administered for six months. However, there was no improvement.

About ten months after onset, muscle weakness emerged in the trunk and upper limbs, and non-invasive positive pressure ventilation was initiated. At that time, neurological examination demonstrated lower-limb dominant tetraparesis and absence of the tendon reflex in the bilateral lower limbs. There was no facial palsy, dysarthria, dysphasia, or tongue atrophy. Nerve conduction study demonstrated marked reduction of the amplitude of the CMAPs in the four limbs (< 0.3mV), but conduction velocity remained within normal limits. Muscle action potentials were not detected in the

right tibial nerve or left ulnar nerve. Sensory nerve action potentials (SNAPs) were within normal limits. The patient refused artificial respiratory support and died of respiratory failure 13 months after onset. There were no symptoms suggesting bladder or rectal dysfunction throughout the clinical course. Genetic sequencing could not be performed.

Case II-6

This patient was also diagnosed as having ALS and died at age 39, but information other than this could not be obtained.

3. Methods

Brain and spinal cord tissue samples of case II-4 were fixed postmortem with 10% formalin and embedded in paraffin. Ten-micrometer-thick (Multiple 10-μm-thick) sections were prepared from the cereberum, midbrain, pons, medulla oblongata, cerebellum, and spinal cord including the cervical, thoracic, lumbar and sacral segments. These sections were stained with hematoxylin–eosin (HE) and Klüver–Barrera (KB) and by the Bodian impregnation method. Spinal cord sections were examined immunohistochemically by the immunoperoxidase method using 3,3'-diaminobenzidine tetrahydrochloride and hematoxylin as the chromogen and counterstain, respectively. Antibodies used in this study are shown in Table 1.

4. Results

4. 1. Neuropathological findings

Brain weight was 1225 g after fixation. Macroscopically, there were no abnormalities in the brain. The anterior roots of the spinal cord were atrophic. Microscopically, as previously reported in

ALS cases showing SOD1 mutation, the posterior funiculus was involved throughout the whole spinal cord, and the posterior cerebellar tract was also affected in the thoracic and cervical cord (Fig. 3A, B). In the thoracic cord, neurons of Clarke's nucleus were depleted, whereas the intermediolateral nucleus was preserved. The CST involvement was not apparent by KB staining (Fig. 3A). Severe neuron loss was observed in the anterior horn of the whole spinal cord (Fig. 3C). The number of neurons in Onuf's nucleus were slightly decreased on the right side, and markedly decreased on the left (Fig. 3D). This finding was confirmed on ten serial sections. Neuronal hyaline inclusions were rarely observed in the remaining neurons of the spinal cord anterior horn including Onuf's nucleus (Fig. 3E). There were no apparent astrocytic hyaline inclusions [13]. In the brainstem, neuronal loss was slight in the hypoglossal nucleus, and was not apparent in the motor nucleus of the trigeminal nerve. There were no Bunina bodies. There was no apparent neuronal loss in the cerebrum or cerebellum including the primary motor cortex, although the neurons in these regions frequently showed ischemic changes related to hypoxia in the terminal stage of the disease.

4. 2. Immunohistochemical findings

Although the CST involvement was not apparent by myelin stain as described above, CD68 immunohistochemistry demonstrated proliferation of activated macrophages/microglia in the CST (Fig. 3B). CD68 immunohistochemistry also showed involvement of the posterior cerebellar tract, Clarke's nucleus (Fig. 3B), and inferior cerebellar peduncle of the medulla oblongata. Ubiquitin, p62, and SOD1 immunohistochemistry demonstrated neuronal cytoplasmic inclusions in the remaining neurons of the spinal cord anterior horn including Onuf's nucleus (Fig. 3F-H). Immunoreactivity for neurofilament or TDP-43 was not apparent in the inclusions. Onuf's nucleus sections containing hyaline inclusions were first stained with HE, and photographed, and then destained in ethanol, and

finally immunostained for SOD1. Hyaline inclusions in the remaining neurons of Onuf's nucleus identified by HE staining (Fig. 3E) were partially and irregularly imunoreactive for SOD1 (Fig. 3F).

5. Discussion

The clinical features of FALS with Gly72 Ser mutation in the SOD1 gene is summarized in Table 2. Unlike previous reports [16, 17], our cases II-2 and II-4 showed disease onset in the sixth or seventh decade. In addition, case II-2 developed weakness of the upper limbs, and the lower limbs were well preserved throughout the clinical course. These findings indicate that the same Gly72Ser mutation may present different clinical courses, even within the same family. In case II-4 (autopsied patient), neurological examination had shown decreased vibration sensation in the lower limbs although the patient did not complain of sensory symptoms. Additionally, we could not clinically exclude the possibility of CIDP in this patient because the upper motor neuron signs were limited. In the view of clinicopathological correlation, impaired vibration sensation may be due to posterior funiculus involvement, and limited upper motor neuron signs appeared to reflect mild CST involvement.

Neuropathologically, many SOD1-mutated FALS cases show neuronal Lewy body-like hyaline inclusions. In addition, long surviving FALS patients with SOD1 gene mutations present with astrocytic hyaline inclusions [7]. These neuronal and astrocytic inclusions contain both wild-type and mutant SOD1 protein. In some mutations, however, SOD1 aggregation is not demonstrated histopathologically [7], therefore the process of neuron death may somewhat differ among the mutations. Further study is required to clarify the process of neuronal degeneration in ALS with SOD1 mutaion.

The salient pathological feature in case II-4 was neuron loss in Onuf's nucleus, although

clinical symptoms suggesting bladder or rectal dysfunction were absent. SOD1 aggregation demonstrated in the remaining neurons of Onuf's nucleus suggests that Onuf's nucleus was involved in the disease process associated with Gly72Ser mutation. To date, Onuf's nucleus has been considered largely intact in ALS [10, 11], and neuron loss has hardly been seen except in patients showing prolonged disease duration with artificial respiratory support [12-15]. Exceptionally, Yoshida et al [18] and Kihira et al [19] reported a FALS case and a SALS case respectively, showing neuron loss in Onuf's nucleus despite disease duration shorter than three years. In the other study, Kihira et al. showed Bunina bodies and ubiquitin-immunoreactive inclusions in the neurons of Onuf's nucleus in some ALS cases while neuron loss was not apparent [20]. They concluded that Onuf's nucleus is involved in the disease process in some ALS cases, although the severity of degeneration was of a lesser degree than that in other motor nuclei. Among ALS cases showing the SOD1 mutation, neuron loss with neuronal and glial inclusions in Onuf's nucleus was reported only in one patient with two base pair deletion in codon 126 of exon 5 [13], who showed a prolonged disease duration with artificial respilatory support.

In conclusion, the findings in this family provide new information regarding the clinicopathological features of FALS with Gly72Ser mutation in the SOD1 gene. Further clinicopathological information in a larger number of cases showing Gly72Ser mutation is needed to clarify whether involvement of Onuf's nucleus is associated with this mutation.

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Table 1 Antibodies used for immunohistochemistry

Antibody	Type	Sourse	Dilution
Anti-ubiquitin	Rabbit polyclonal	Dako, Glostrup, Denmark	1:2000
Anti-p62 (SQSTM1)	Rabbit polyclonal	Biomol	1:1000
Anti-TDP-43	Rabbit polyclonal	Proteintech	1:1000
Anti-SOD1	Rabbit polyclonal	Made by Asayama et al. [21]	1:20000
Anti-neurofilament (SMI 31)	Mouse monoclonal	Sternberger, Lutherville, MD, USA	1:1000
Anti-CD68	Mouse monoclonal	Dako, Glostrup, Denmark	1:100

Table 2 Clinical features of familial amyotrophic lateral sclerosis with Gly72Ser mutation in the SOD1 gene

	Age at onset /Gender	Age at death	Site of onset	Atypical features	Disease penetrance in the family
Orrell et al. III-3 [16]	47/M	51	Right foot	Decreased vibration sensation, No UMN signs	Incomplete
III-8 [16]	46 or 47/F	49	Legs	ND	
Shaw et al. [17]	29/M	30 or 31	Left thigh	No family history	Incomplete
Present case II-2	75/M	76	Upper limbs	ND	Incomplete
case II-4	66/F	67	Left thigh	Decreased vibration sensation, Limited UMN signs	
case II-6	ND/M	39	ND	ND	

M male, *F* female, *UMN* upper motor neuron, *ND* not described,

Figure legends

Fig. 1. Pedigree of a Japanese family with FALS harboring Gly72Ser mutation in the SOD1 gene. Males are represented by square, females by circles. Affected members are represented by solid symbols, deceased individuals by diagonals.

Fig. 2. Direct sequencing of SOD1 gene exon 3 showed missense mutation substituting glycine for serine (Gly72Ser).

Fig. 3. (A) and (B) are serial sections. A. In the lower thoracic cord, the central portion of the posterior funiculus was involved with left predominance, and the posterior intermediate sulci could not be identified. Although the subpial region was not well stained by KB staining, myelin pallor was evident in the posterior cerebellar tract (arrowhead). The corticospinal tract (CST) involvement was not apparent (arrow). B. CD68 immunohistochemistry of the left lateral and posterior funiculi showed proliferation of activated macrophages/microglia in the CST, Clarke's nucleus (arrowhead), posterior cerebellar tract and posterior funiculus. In the posterior funiculus, the region around the posterior central sulcus (arrow) was almost intact. C. Marked neuronal loss was observed in the anterior horn of the left second sacral segment. The arrow indicates Onuf's nucleus. D. A high-power view of the left Onuf's nucleus (arrowheads) demonstrated severe neuron loss. E, F. The hyaline inclusion (E, arrow) in a neuron of Onuf's nucleus demonstrated by HE staining was partially immunoreactive for SOD1 (F). The nucleus was located in the periphery. G. The left Onuf's nucleus is shown. Using p62 immunohistochemistry, the inclusion in the remaining neuron of Onuf's nucleus were labeled. H. The other inclusion immunoreactive for SOD1 in the remaining

neuron of the anterior horn of the second sacral segment. Scale bars = 1mm (A), 500 μm (C), 200 μm (D), and 50 μm (E-H).