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A Japanese ALS6 family with mutation R521C in the *FUS/TLS* gene: A clinical, pathological and genetic report☆

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ABSTRACT

Here we report a Japanese family with amyotrophic lateral sclerosis (ALS) characterized by very rapid progression, high penetrance and an autosomal dominant mode of inheritance. The phenotype includes atrophy of sternocleidomastoideus muscles, bulbar involvement, weakness of neck muscles and proximal muscle atrophy. These clinical symptoms are reminiscent of myopathy. All patients examined had similar clinical symptoms, age at onset and disease duration. The proband was found to have mutation R521C in the *FUS/TLS* gene, and was diagnosed as having ALS6. Autopsy material was available from the mother of the proband and FUS-immunoreactive neuronal and glial cytoplasmic inclusions were observed in the anterior horn of the spinal cord. While atrophy and weakness of the sternocleidomastoideus muscle is not emphasized in previous reports, this symptom may be a clinical hallmark of ALS6.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, for which no effective therapy is available. It is characterized by generalized skeletal muscle weakness and atrophy due to degeneration of upper and lower motor neurons [1,2]. Only a small proportion of ALS cases (5–10%) have a family history of the disease, and this subset of patients is classified as familial ALS (FALS). To date, a number of genetic loci and disease causing mutations have been identified in FALS patients [3]. Mutations in Cu/Zn superoxide dismutase (*SOD1*) give rise to ALS1 [4,5], whereas mutations in *FUS/TLS* [6,7], *VAPB* [8], *ANG* [9] and *TDP-43* [10,11] give rise to ALS6, ALS8, ALS9 and ALS10, respectively. So far gene mutations have not been identified for ALS3 [12] or ALS7 [13] which show typical ALS phenotypes [14]. Other genes are responsible for forms of familial ALS that show distinctive clinical features from those of typical ALS such as juvenile onset, slow progression, spastic paraparesis, or dementia [15–19]. Here we report a Japanese family with autosomal dominant familial ALS whose clinical features are almost identical between individuals. Of note, proximal muscles are more severely affected than distal ones, and atrophy of the sternocleidomastoideus muscles and weakness of the neck muscles is obvious, reminiscent of myopathy. However they conformed to the El Escorial criteria of ALS and the proband was found to have mutation R521C in the *FUS/TLS* gene. Furthermore neuronal and glial cytoplasmic FUS-positive inclusions were observed in the anterior horn of the spinal cord. The present report clearly shows ALS6 cases occur in Japan, and the clinical and pathological features are atrophy of sternocleidomastoideus muscle, bulbar signs, weakness of the neck muscles, proximal muscle atrophy, rapid progression and FUS-immunoreactive neuronal and glial cytoplasmic inclusions in the anterior horn of the spinal cord.

2. Case report

Clinical features along with biochemical and physiological measurements are summarized for three members of a Japanese pedigree in Table 1.

Abbreviations: SOD1, Cu/Zn superoxide dismutase; ALS, amyotrophic lateral sclerosis.

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Table 1
Clinical data on the present ALS6 family.

	Patient 1 (II-3)	Patient 2 (II-1)	Patient 3 (I-4)
Sex	Female	Male	Female
Age at onset (yr)	38	32	29
Survival period (mo)	26/110[a]	7	15
Intellectual disturbance	–	–	–
Site of onset	Bulbar	Lower limbs	Lower limbs
Mechanical ventilation	+	–	–
Creatine kinase	170/127[b] (normal 20–110 IU/l)	66 (normal 0–170 mU/ml)	NE
Total cholesterol (mg/dl)	254/211[b]	154	NE
High-density lipoprotein cholesterol (mg/dl) 40		NE	NE
Serum hexosamidase A activity	Normal	NE	NE
Cerebrospinal fluid findings			
Total cell count (/μl)	<1	<1	0
Protein (mg/dl)	22	48	Not quantified
Sugar (mg/dl)	64	58	71
NCS (motor and sensory)	Normal	Normal	NE
Needle EMG (all limbs)	Neurogenic	Neurogenic	Neurogenic

–, absent; +, present; NE, not examined; NCS, nerve conduction study; EMG, electromyogram.
[a] The numbers show interval between the onset of symptoms and beginning of ventilator support, and total disease duration, respectively.
[b] The values of these items were examined twice.

2.1. Patient 1

A 38-year-old Japanese female (proband, Subject II-3 in Fig. 1) had difficulty in excreting sputa. She soon developed difficulties eating and exhibited fatigue when chewing. At almost the same time, she developed dysarthria, gait disturbance and had difficulty in raising her left arm. She began to repeatedly fall down and at the age of 39 she could no longer stand up from the sitting position without assistance. Thereafter difficulty with excreting sputa, gait disturbance and weakness in chewing worsened and she was admitted to our hospital. On physical examination, she was alert, found to be slightly obese and had hypertension. Examination of the optic fundi showed findings of hypertension and atherosclerosis, but no optic atrophy or retinal degeneration. Her mental status was normal, and her verbal, performance, and full-scale scores of Wechsler Adult Intelligence Scale were 93, 107 and 99, respectively. Although her ocular movements were normal, she had atrophy and weakness in the masseter muscles. She also had a nasal voice, dysphagia, severe atrophy and weakness in the bilateral sternocleidomastoideus muscles, and atrophy and fasciculation in the tongue. She had proximally dominant muscle atrophy and weakness in all limbs, and muscle tonus was generally decreased. She could not flex her neck in the supine position due to very severe weakness in the neck muscles. Scores of manual muscle testing (MMT) of the flexor and extensor muscles of the neck were 2/5 and 4/5, respectively. She had to walk her hands up her legs in order to stand from a kneeling position (Gowers sign (+)). Snout, jaw, and palmomental reflexes were positive. The deep tendon reflexes were slightly increased in the upper limbs and slightly decreased in the lower limbs. The plantar responses were bilaterally extensor. She had very mild hypalgesia and hypesthesia in her left thigh and lower legs, whereas she had neither rectal and bladder disturbance nor cerebellar signs.

The serum levels of creatine kinase and total cholesterol were slightly elevated as shown in Table 1. Brain computed tomogram and magnetic resonance image were normal. Studies on somatosensory evoked potentials showed normal findings although some theta but not epileptiform activity was present in the electroencephalogram. Muscle biopsy was performed in the left quadriceps and the specimens displayed small angulated fibers and fiber type grouping. Thereafter her clinical symptoms progressed very rapidly, and she required ventilator support approximately 26 months after the onset of symptoms. By the age of 45, she showed very severe atrophy and weakness in general skeletal muscles, but her ocular movements were preserved and communication was possible with eye movement. Subsequently she had repeated pneumonia, and died of respiratory failure at the age of 47. An autopsy could not be performed.

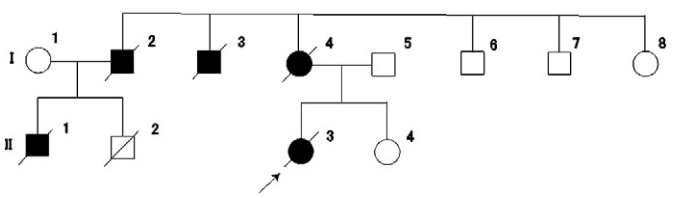

Fig. 1. Pedigree tree of a Japanese family with ALS6.

2.2. Genetic analysis

Blood samples with informed consent were obtained from this patient, and 73 normal controls, and genomic DNA was isolated by a standard phenol/chloroform method. The five exons of SOD1, the six exons of VAPB, and the single coding exon of angiogenin, including exon–intron boundaries, were amplified by polymerase chain reaction (PCR) and sequenced as previously described [8,9,20,21]. No mutation was identified in any of these genes. Exons 1, 9, 10, 11, 12 and 13 of MAPT (TAU) were also screened, but no mutation was detected. According to a published method [7], the 15 exons of the *FUS/TLS* gene were amplified by PCR, and directly sequenced. For reproducible sequencing results, exon 15 was sequenced using an internal reverse primer, 5-cttgggtgatcaggaattgg-3. As a result, a disease-causing mutation, c.1561 C>T (R521C) was detected in exon 15 in the *FUS/TLS* gene (Fig. 2). To rule out the possibility of sequencing errors, the PCR products from exon 15 were subcloned into the TA vector (Invitrogen, Carlsbad, CA) and sequenced. The missense mutation was also confirmed by AlwI restriction digest of PCR product (344 bp) (New England Biolabs, Tokyo, Japan). Wild-type allele when digested produces a 218- and 126-bp fragment, whereas the mutant allele is not cut. This PCR-restriction fragment length polymorphism (RFLP) analysis confirmed patient 1 was heterozygous for the wild-type and mutant allele with both cut and uncut products being observed (Fig. 3). Of 72 normal Japanese controls subjected to RFLP, all showed homozygosity for the wild-type allele. Two synonymous polymorphisms, c.147 C>A in exon 3 and c.291 C>T in exon 4 were also detected in this patient.

2.3. Patient 2

Patient 2 (Subject II-1 in Fig. 1), a 32-year-old male, showed gait disturbance, and gradually developed dysarthria. Approximately