ORIGINAL ARTICLE

Marked intrafamilial phenotypic variation in a family with SOD1 C111Y mutation

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Abstract

Objectives: To identify the disease-causing mutation in and report on the clinical features of a Japanese family that had coexisting phenotypes of amyotrophic lateral sclerosis and spinal muscular atrophy. **Methods:** The family comprised 9 patients (6 men and 3 women). We reviewed their clinical records and performed mutation analysis of the copper/zinc superoxide dismutase (*SOD1*) gene in some of these patients. **Results:** The patients either had a rapid (n = 7) or an extremely long (n = 2) clinical course. The mean age at onset was 39.0 ± 13.7 years (range: 20–68 years). The initial symptoms were bulbar palsy (n = 2), upper (n = 4) or lower (n = 2) limb muscle weakness, or leg cramps (n = 1). The total disease duration varied widely, ranging from 1 year to >69 years. We identified a SOD1 C111Y mutation in the patients of this family. **Conclusion:** The family showed a marked intrafamilial phenotypic variation associated with the SOD1 C111Y mutation. Elucidating the biological basis of disease expression in patients with SOD1 C111Y mutation may provide us with useful information to develop therapeutic approaches and to prevent disease progression.

Key words: *familial amyotrophic lateral sclerosis, spinal muscular atrophy, copper/zinc superoxide dismutase (SOD1), gene analysis, slow progression*

Introduction

Amyotrophic lateral sclerosis (ALS) is a rapidly progressing, neurodegenerative disorder characterized by the selective loss of upper and lower motor neurons. Approximately 10% of ALS cases are familial (FALS) (1), and 20% of cases among FALS families are caused by mutations in the copper/zinc superoxide dismutase (*SOD1*) gene (referred to as ALS1) (2, 3). To date, >150 mutations in the *SOD1* gene have been identified among ALS1 patients (4). Patients having *SOD1* mutations show varied clinical phenotypes in terms of age at onset, site of onset, and disease duration within or between families (5).

We previously described a large family with coexisting phenotypes of ALS and spinal muscular atrophy (SMA) (6). We recently identified a SOD1 C111Y mutation in this family. Two patients with the SOD1 C111Y mutation were previously reported to exhibit the typical ALS clinical characteristics and course (7, 8). Here, we report peculiar characteristics in patients with this mutation, implying the existence of modifying factors that can delay or prevent progression of ALS1.

Methods

Subjects

This familial case—involving a 4-generation family including 7 patients showing a rapid (n = 7) or very slow (n = 2) clinical course (**Fig. 1**)—was previously reported by Nakano et al. (12), and the pedigree has been updated in our hospitals. We reviewed these patients' clinical records and reevaluated their phenotype using the revised El Escorial-Awaji criteria (9).

Mutation analysis in the *SOD1* gene

After informed consent was obtained, genomic DNA of patients III-4 and IV-6 was extracted from peripheral whole blood. It was also extracted from a paraffin block of the autopsied liver from patient III-5, using a WaxFree^{TM} DNA kit (TrimGen, Sparks, MD, USA). Exons 1–5 of the *SOD1* gene were amplified by polymerase chain reaction (PCR) (10) and then analyzed by direct sequencing. Sequence variation was assessed by restriction fragment length polymorphism (RFLP) analysis with digestion with *Hpy*CH4V, followed by agarose gel electrophoresis.

Results

Patient characteristics

The clinical characteristics of the 9 patients (6 males and 3 females) are summarized in **Table 1**. The mean age at onset was 39.0 ± 13.7 (range 20–68) years, and the disease duration varied markedly, from 1 to >69 years. The initial symptoms were bulbar palsy (n = 2), muscle weakness of upper (n = 4) or lower (n = 2) limbs, or leg cramps (n = 1). Fasciculation was observed in 8 patients (89%). Exaggerated hyperreflexia was observed in patients IV-6 and IV-8, and positive pyramidal tract signs in patient IV-6. No patient showed sensory impairment upon physical examination, but patient IV-8 showed decreased sensory nerve conduction velocities in the distal left leg. Needle electromyography (EMG) revealed polyphasic and/or high amplitude potentials in the 4 patients examined (II-6, III-4, IV-6, and IV-8). Upper motor neuron signs and electrophysiological abnormalities could not be confirmed from the clinical records of patients II-1, II-2, III-9, and IV-1. Clinically definite ALS was diagnosed in patients IV-6 and IV-8 but could not be diagnosed in patients II-6, III-4, and III-5 without

confirmed upper motor neuron signs. Each representative case with a rapid (III-5) or with an extremely long (III-4) clinical course is described below.

Rapid clinical course

Patient III-5 experienced weakness in his right leg and gait disturbance at age 51. Two months later, he was unable to run or climb stairs. Four months after onset, he was admitted to our hospital for further evaluation. Neurological examination revealed moderate atrophy of his right lower leg muscles, and fasciculation in the tongue and upper proximal and limb-girdle muscles. Left biceps tendon reflex was decreased, but other deep tendon reflexes in the upper and lower limbs and Babinski sign were negative. No sensory and autonomic disturbances were observed. Needle EMG revealed polyphasic potentials in his right upper limb muscles but no potentials in his right lower limb. Motor and sensory nerve conduction velocities were normal in the right ulnar and tibial nerves. Eleven months after onset, he developed atrophy and weakness in his left upper and lower limb muscles and dyspnea, subsequently dying of respiratory failure. The disease duration was 1.2 years.

Extremely long clinical course

Patient III-4 noticed muscle weakness in his upper limbs at age 20 years. His symptoms slowly worsened, and he experienced difficulty lifting heavy materials at age 40. At age 56, he was admitted to our hospital for investigation. Neurological examination revealed mild atrophy, weakness, and fasciculation on the right dominant deltoid, triceps, and biceps muscles. Deep tendon reflexes were decreased in the upper limbs but normal in the lower limbs. His Babinski reflex was negative. No sensory and autonomic nerve

involvement was observed. Blood and cerebrospinal fluid findings were normal. Needle EMG showed polyphasic potentials in his upper and lower limb muscles and high amplitude potentials in his right triceps muscle. He was diagnosed with SMA but was not followed by us for ~20 years. He was admitted to a special elderly nursing home at age 84, and was able to walk at age 87. At 88, he developed pneumonia and dehydration as complications and was transferred to our hospital. His level of consciousness was impaired, but spontaneous respiration was preserved under oxygen administration. Generalized emaciation and diffuse muscular atrophy, except of the tongue, were observed. Computed tomography of whole muscles revealed a decrease in muscle volume (**Fig. 2**). His condition gradually improved after receiving intravenous fluids and antibiotics, and he was able to eat unaided and continue rehabilitation. We re-evaluated nerve conduction velocities and performed needle EMG at age 89. The motor and sensory nerve conduction velocities were almost normal in the left upper and both lower limbs. The EMG demonstrated the presence of long-duration polyphasic potentials suggesting reinnervation in left upper and lower limb muscles and tongue. The disease duration was >69 years.

Mutation analysis of the *SOD1* gene

We discovered a heterozygous mutation c.111 C>G in exon 4 of the *SOD1* gene in patients III-4, III-5, and IV-6 (**Fig. 3A**). This mutation leads to substitution of cysteine (TGC) with tyrosine (TAC) at codon 111 in the SOD1 protein (C111Y). RFLP analysis revealed the heterozygous mutation in patients III-4, III-5, and IV-6, but not in the normal control subjects (**Fig. 3B**).

Discussion

A disease duration of >69 years, observed in patient III-4, is the longest among reported ALS1 cases. In previous studies, patients with the mutations SOD1 H46R (11) and L144F (12) have had extremely long survival times of 47 and 44 years, respectively. In another extreme case, an SPG4 mutation was reported as a clinical variant of ALS (13). Patients II-6 and III-4 most likely have classical Vulpian-Bernhardt syndrome, a clinical variant of ALS (14). These cases are rare, usually have a far more protracted disease course, and have earlier been reported among patients with ALS1. Except for their extreme survival times, the disease features of patients II-6 and III-4 may be slightly inconsistent with this syndrome.

The most distinct feature of the studied family is the coexistence of rapid and extremely slow clinical courses. We have summarized *SOD1* mutation cases with a disease duration of >20 years in **Table 2**. Patients harboring E22G, G37R, H46R, G93D, G93C, or I104F mutations exhibited generally mild clinical courses (11, 15–21). On the other hand, patients with I113T, L144F, and C111Y mutations exhibited intrafamilial phenotypic variation from rapid to slow progression. Although the penetrance of the I113T mutation is reported to range from complete to incomplete (22, 23) and the penetrance of C111Y has not yet been determined, a common mechanism may underlie the pathogenesis of these mutations.

Recent investigations have demonstrated that cysteine residues at codon 111 are critical for mediating disulfide cross-linking and rapidly mediate the aggregation and toxicity of mutant SOD1 (24–27). A C111S mutation strongly reduces the ability of ALS1-associated mutant SOD1 to form aggregates (25). Furthermore, an aberrant Cu interaction with mutant SOD1 is potentially toxic and is implicated in neuronal cell

death in ALS (28). The C111S mutation markedly attenuates the affinity for Cu and increases protein stability. This implies that the Cys111 residue is critical for the stability of mutant SOD1 (29). These hypothetical mechanisms may have influenced the different phenotypes in our patients.

In conclusion, we present a case of marked intrafamilial phenotypic variation in the phenotype associated with a SOD1 C111Y mutation. Understanding the biological basis of this variation may be helpful for developing approaches to treat and prevent disease progression.

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Figure legends

Figure 1

Pedigree of the family. Black: patients with a rapid clinical course, gray: patients with a very slow clinical course, white: unaffected individuals. I–IV refers to the generation.

Figure 2

Muscle computed tomography images in patient III-4. Muscle volume of upper trunk and humeri (**A**), lower trunk and forearms (**B**), thighs (**C**), and lower legs (**D**) was decreased.

Figure 3

(**A**) Sequence analysis of exon 4 of the *SOD1* gene in patient IV-6. A transition of G to A was detected in the mutant allele, resulting in the substitution of cysteine (C) to tyrosine (Y) at residue 111 in the protein.

(**B**) Restriction enzyme (*Hpy*CH4V) fragment length polymorphism analysis in 3 patients presenting with a rapid (III-5 and IV-6) or an extremely slow (III-4) clinical course, as well as a healthy control. Lower panel: The amplification size of a normal PCR product of exon 4 is 236 bp. There are 2 *Hpy*CH4V restriction sites in the normal product. When the normal product is digested by *Hpy*CH4V, the fragment sizes are 134 bp, 45 bp, and 57 bp. The codon 111 mutation (C111Y) disrupts one of the enzyme's restriction sites, resulting in fragment sizes of 179 bp and 57 bp. All patients had both normal and mutant alleles. M, molecular weight marker; C, normal control.

Table 1. Clinical characteristics of patients in a family harboring C111Y mutation in the *SOD1* gene

Case	Sex	Age at onset (years)	Age at death (years)	Disease duration (years)	First neurological symptom	Tongue atrophy	Fasciculation	DTR upper lower	Pyramidal signs	Neurophysiological study results
II-1	F	68	69	1.0	Bulbar palsy	N/A	N/A	N/A N/A	N/A	N/A
II-2	F	33	35	2.0	Bulbar palsy	(+)	N/A	N/A N/A	N/A	N/A
II-6	M	30	78	48	Weakness in the upper limbs	(−)	(+)	N ↓	(−)	EMG: high amplitude potential at biceps brachii, polyphasic potential in the lower leg muscles
III-4	M	20	>89 (still alive)	>69	Weakness in the upper limbs	(−)	(−)	↓ N	(−)	At age 53 years: EMG: diffuse polyphasic potential in the right upper and lower limb muscles, and high amplitude in the right triceps brachii At age 89 years: MCV: N in left upper limb and both lower limbs SCV: left upper limb and both lower limbs EMG: long-duration polyphasic potential in upper and lower limb muscles, and tongue
III-5	M	51	53	1.2	Weakness in the right leg	(−)	(+)	(−)/↓ (−)	N/A	MCV: N in right ulnar and tibial nerves SCV: N in right ulnar and tibial nerves EMG: polyphasic potentials in right upper limb muscles but no potential activity in right lower limb muscles
III-9	F	30	31	1.0	Weakness in the lower limbs	N/A	N/A	NA NA	N/A	N/A
IV-1	M	36	38	2.0	Weakness in the left upper limb	(−)	(+)	N N~↓	(−)	N/A
IV-6	M	49	53	4.0 (tube feeding)	Weakness in the right hand	(+)	(+)	↑ N	(+)	MCV: N SCV: N EMG: fibrillation potential, fasciculation potential, high amplitude
IV-8	M	34	34	1.0	Cramp in the left leg	(+)	(+)	↑ ↑/↓	(−)	MCV: N SCV: decrease in left distal leg EMG: polyphasic potential and giant potential
Range		20–68	31–>89	1.0–>69						

SD, standard deviation; N/A, data not available; N, normal; DTR, deep tendon reflex; EMG, electromyography; MCV, motor nerve conduction velocity; SCV, sensory nerve conduction velocity

Table 2. Comparison of clinical profiles of families having a SOD1 mutation with disease duration >20 years

SOD1 mutation	No. of patients	Age at onset (years)		Disease duration (years)			Penetrance	Marked intrafamilial phenotypic variation	Site of onset	Upper motor neuron signs	Reference No.
		Mean (± SD)	Range	Mean (± SD)	Median	Range					
E22G	15	51.8 (10.2)	38~71	13.2 (8.6)	13	2~>29	N/A	(−)	Upper or lower limb	(±)	18
G37R	5	36.5	N/A	24.5	N/A	N/A	High	(−)	Lower limb	(+)	19
H46R	10	49.6 (10.9)	N/A	17.3 (10.7) (n = 4)	N/A	~>28	Complete	(−)	Lower limb	(±)	16
	13	49.6 (10.9) (n = 10)	NA	15.8 (9.9) (n = 5)	NA	N/A	Complete	(−)	Upper or lower limb	(±)	17
	14	48.0 (9.5)	N/A	16.8 (6.8) (n = 9)	N/A	N/A	Complete	(−)	Upper or lower limb	(±)	17
	15	39.7 (10.5) (n = 9)	N/A	18.1 (13.2) (n = 9)	N/A	~>47	Complete	(−)	Lower limb	(−)	11
	15	42.9 (4.7) (n = 7)	N/A	17.8 (8.1) (n = 7)	N/A	~23	Complete	(−)	Lower limb	(±)	15
G93C	20	45.9 (10.6)	33~71	13 (4)	12.8	5~20	Complete	(−)	Lower limb	(−)	20
G93D	3	53.7 (15)	45~71	10 (10.4)	4	4~>22	Incomplete	(±)	Upper or lower limb	(−)	21
I104F	5	33.0 (20.7) (n = 4)	6~55	21.3 (11.8) (n = 3)	NA	12~38	Incomplete?	(−)	Upper or lower limb	(+)	17
C111Y	9	14.4 (25.6)	20~69	2 (8.5)	2	1~>69	Complete?	(+)	Upper or lower limb or bulbar	(±)	Present study
I113T	5	59 (n = 2)	48~70?	7.5 (9.1) (n = 4)	3.5	2.5~21	Incomplete	(+)	Lower limb	(±)	23
L144F	2	49.5	28~71	22.8	22.8	1.6~44	N/A	(+)	Lower limb	(+)	12

N/A, data not available

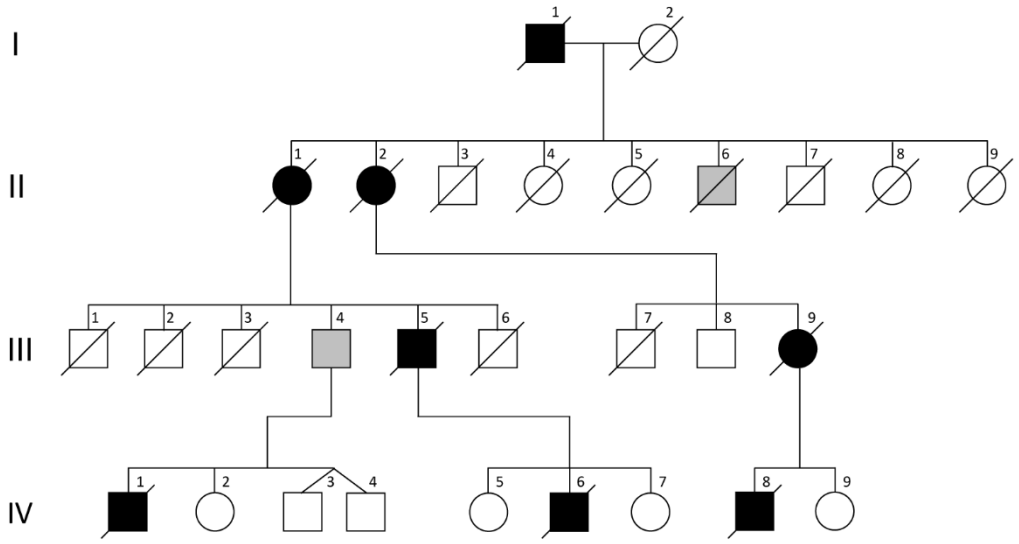

Figure 1

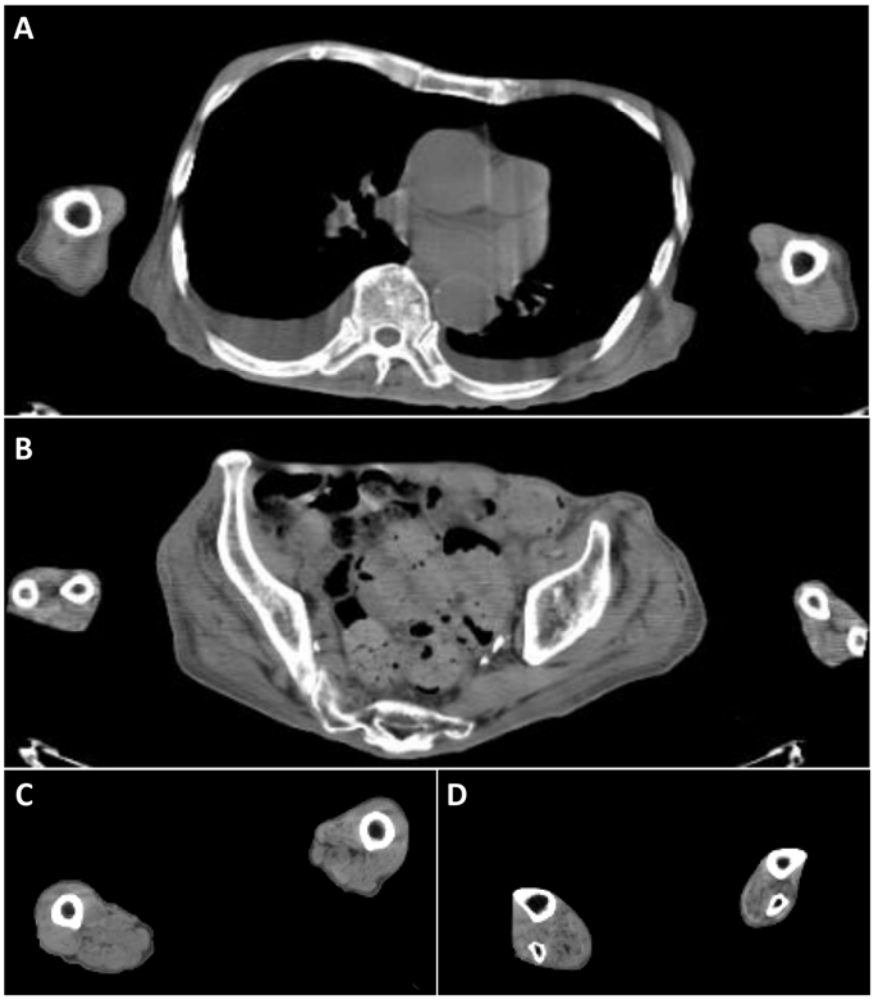

Figure 2

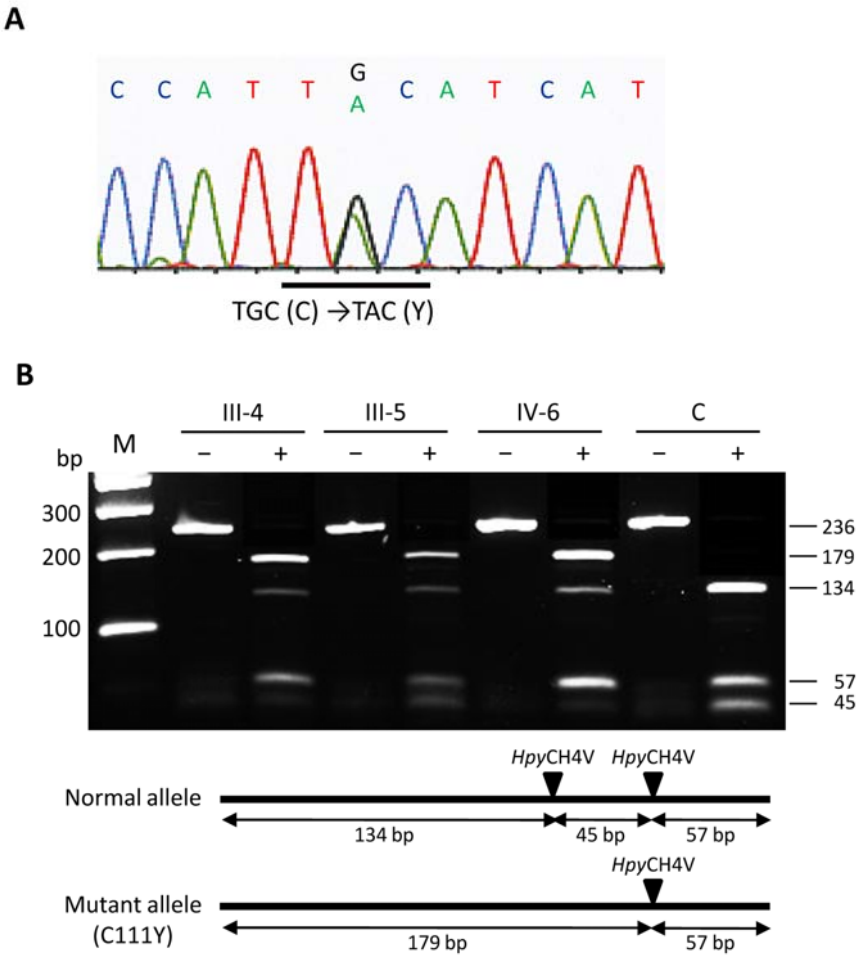
A
C C A T T G/A C A T C A T
TGC (C) →TAC (Y)
B
III-4
III-5
IV-6
C
M
bp
300
200
100
236
179
134
57
45
HpyCH4V
HpyCH4V
Normal allele
134 bp
45 bp
57 bp
HpyCH4V
Mutant allele
(C111Y)
179 bp
57 bp

Figure 3