Anticipation and Phenotypic Heterogeneity in Korean Familial Amyotrophic Lateral Sclerosis with Superoxide Dismutase 1 Gene Mutation

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Background and Purpose: Different mutations in the Cu/Zn superoxide dismutase 1 (*SOD1*) gene have been reported in approximately 10% of cases of familial amyotrophic lateral sclerosis (ALS). The aim of this study was to analyze for mutations in the *SOD1* gene and clinical characteristics in Korean family of ALS.

Methods: A subpopulation of the family reported here has been described previously. In the present study, we analyzed the *SOD1* gene in the proband and his immediate family members, who were not reported on previously. Genomic DNA was isolated from the leukocytes of whole blood samples and the coding region of the *SOD1* gene was analyzed by PCR and direct sequencing.

Results: The genetic alterations were a GGC-to-GTT transition at codon 10 in exon 1 and [IVS4+15_16insA; IVS4+42delG; IVS4+59_60insT] in intron 4. Patients with these mutations exhibit diverse clinical onset symptoms and acceleration of the age at onset in successive generations, which is called anticipation.

Conclusions: We have described a family with familial ALS that showed autosomal-dominant inheritance and two distinct genetic alterations in *Cu/Zn-SOD1*. The affected family members had different phenotypes and anticipation.
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Key Words : Familial amyotrophic lateral sclerosis, Superoxide dismutase 1 (*SOD1*) gene, Anticipation

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disorder characterized by the death of motor neurons in the cerebral cortex, brainstem, and spinal cord. ALS occurs in both familial and sporadic forms.[1] Familial ALS (FALS) represents 5-10% of ALS cases, and is usually expressed as an autosomal-dominant trait that is clinically indistinguishable from sporadic cases. Many such familial cases have been reported to be associated with mutations in the gene that codes for Cu/Zn superoxide dismutase (*SOD1*).[2,3]

More than 122 mutations of the *SOD1* gene have been reported in FALS and sporadic ALS patients,[4] the vast majority of which are point mutations (single-base-pair substitutions) that are widely distributed within the gene, through exons 1-5. It is well known that the sites

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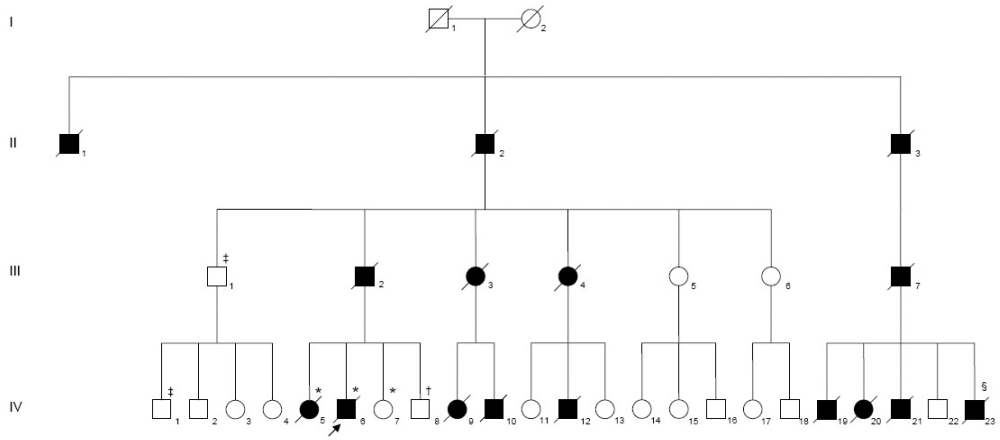

Figure 1. Pedigree of the family. Squares represent males and circles represent females. Filled symbols represent those affected with ALS. Oblique lines represent deceased family members. The arrow indicates the proband. Each mark represents the result of genetic testing as follows:
* indicates carriers of the heterozygous G10V mutation plus intronic alterations of the *SOD1* gene.
† indicates an individual who tested positive for the intronic alterations alone.
‡ indicates individuals who showed normal sequences of the *SOD1* gene.
§ indicates an individual who has been previously described as being positive for the G10V mutation.[15]

and types of mutations associated with ALS are not correlated with clinical findings such as age at onset and duration of illness.[5] A few frameshift and nonsense mutations have also been reported in cases of FALS as well as sporadic ALS,[6-13] and have been found predominantly in exon 4, intron 4, and exon 5.[14]

A subpopulation of the family reported here has been described previously.[15] However, since the proband and his immediate family were not included in the previous report, in this study we analyzed the *SOD1* gene in this family group, and found genetic alterations in exon 1 and intron 4 that have not been previously described.

PATIENTS AND METHODS

1. Patients

The pedigree of the family is shown in Figure 1. After obtaining their informed consent, we studied 6 family members from a 4-generation pedigree with 16 members affected by FALS. The proband in this study (individual IV-6), a 37-year-old male, was admitted to a hospital in July 2002 with rapidly progressing hoarseness and subjective weakness in all four limbs (he died 7 months later). We were able to obtain information on the family pedigree. The age at onset of disease was designated as the first sign of weakness, atrophy, or clinical symptoms involving upper- or lower-motor neurons. ALS was diagnosed using the El Escorial criteria as suggested by the World Federation of Neurology Research Group on Neuromuscular disorders.[16] The information pertaining to family members for whom no clinical records were available was obtained from other family members; this information was useful for fully understanding the modality of genetic transmission.

The study was approved by the local ethics committee, and all patients gave their written informed consent for participation.

2. Molecular genetic analysis

The presence of *SOD1* mutations was analyzed in the affected proband and five family members. Genomic DNA was isolated from blood leukocytes obtained from each individual using the QIAamp DNA Blood Mini Kit (QIAGEN, CA, USA). The region encoding the five

Table 1. Age at disease onset and disease duration in parent and offspring generations (mean±SD values)

	Age at disease onset (years)			Disease duration (months)		
Generation	Males (*n*=11)	Females (*n*=5)	Total	Males (*n*=11)	Females (*n*=5)	Total
II	52.7±4.7	-	52.7±4.7	14.4±3.6	-	14.4±3.6
III	43.0±2.8	47.5±0.7	45.3±3.1	18.0±8.4	13.2±1.2	15.6±6.0
IV	29.7±7.6	39.0±2.6	32.8±7.7	13.2±6.0	15.6±3.6	13.2±4.8

exons of the *SOD1* gene were amplified from genomic DNA with the polymerase chain reaction (PCR) using previously described primers.[11] The reactions were performed in 5 μl volumes, and included 100 ng of template (genomic DNA), each primer at 10 pmol/μl, 2.5 mM dNTP, and 1 U of Taq polymerase (Perkin-Elmer, MA, USA) in the buffer with $MgCl_2$. PCR amplification was performed using 30 cycles of 1 min at 94℃, 45 sec at 60℃, and 1 min at 72℃. The resulting PCR products were sequenced using the ABI Prism BigDye terminator cycle sequencing Ready Reaction kit (PE Applied Biosystems, CA, USA) and forward and reverse primers used in the PCR amplification. The sequencing reaction was performed with the ABI 3700 automated sequencer (PE Applied Biosystems). The data were collected and analyzed using ABI DNA sequence analysis software (version 3.6).

RESULTS

1. Clinical findings

The proband (IV-6), a right-handed 37-year-old male, is identified by the black arrow on Figure 1. He first noticed hoarseness and subjective weakness in all limbs at 36 years of age. A standard medical workup did not reveal any abnormalities. A neurological examination showed mild weakness of all four limbs with muscular atrophy and hyperactive deep tendon reflexes. Bulbar symptoms such as dysarthria, dysphagia, and tongue fasciculations were also present.

His motor and sensory nerve conduction velocities were normal. Electromyography revealed increased insertional activity, as well as active denervation discharges in three paraspinal regions (cervical, thoracic, and lumbosacral), and fasciculation potentials in all limbs.

2. Family analysis

The pedigree consisted of 35 members, distributed throughout 4 generations. Sixteen affected members were identified: three patients on the basis of anamnestic data (probable disease), five patients that have been previously reported,[15] and eight patients after review of the available medical records (cases of diagnosed disease). There were no skipped generations.

The pedigree revealed the presence of autosomal-dominant transmission. The age at onset was 39.3±10.6 years (mean±SD; range, 20 to 58 years) in diagnosed family members with probable disease (ages at onset were 52.7±4.7, 45.3±3.1, and 32.8±7.7 years in generations II, III, and IV, respectively; $p<0.01$ by Kruskal-Wallis test) (Table 1). However, the duration of disease symptoms (14.7±5.3 months; range, 7–24 months) did not differ among individuals from this pedigree. The initial clinical symptoms varied among the family members (Table 2). In contrast to the previous report (on individuals III-19, -20, -21, and -23),[15] the proband (III-6) and his elder sister (III-5) showed bulbar symptoms including hoarseness, swallowing difficulty, and respiratory difficulty.

3. Molecular genetic analysis

DNA analysis of the proband (IV-6) revealed the presence of a heterozygous GGC-to-GTT mutation resulting in a substitution of valine for glycine at codon 10 in exon 1 and non-amino-acid-altering variations [IVS4+15_16insA; IVS4+42delG; IVS4+59_60insT] in intron 4 of the *SOD1* gene (Fig. 2). The same changes were also detected in two sisters (IV-5 and IV-7). The

Table 2. Clinical manifestations in family members

Patient designation	Sex	Age at onset (years)	Duration (time to death, months)	First neurologic symptom noticed by patient	Neurologic findings described by physicians at the first examination	EMG findings
II-1	Male	49	24	Hoarseness*	NA	NA
II-2	Male	58	18	Weakness of the lower limbs*	NA	NA
II-3	Male	51	12	Hoarseness and respiration difficulty*	NA	NA
III-2	Male	45	24	Hoarseness and swallowing difficulty	Atrophied general musculature and respiration difficulty	NA
III-3	Female	47	12	Hoarseness and swallowing difficulty	Weakness of the distal muscles on the extremities and swallowing difficulty	NA
III-4	Female	48	12	Weakness of the upper and lower limbs	Gait disturbance and weakness of the lower extremities	NA
III-7	Male	41	12	Weakness of the limbs	Swallowing difficulty, dysarthria, respiration difficulty, and weakness of the limbs	NA
IV-5	Female	40	18	Hoarseness and respiration difficulty	Fasciculation and atrophy of the tongue, weakness of the distal muscles on the extremities, hyperactive DTR, and no Babinski sign	Consistent
IV-6	Male	36	10	Hoarseness and swallowing difficulty	Fasciculation and atrophy of the tongue, atrophy and weakness of the distal muscles on the extremities, hyperactive DTR, and no Babinski sign	Consistent
IV-7	Female	-	Still alive	No symptom during a 5-year follow-up	Only hyperactive DTR	Inconsistent
IV-8	Male	-	Still alive	No symptom during a 5-year follow-up	Normal	Inconsistent
IV-9	Female	41	12	Weakness of the lower limbs	Reduced strength of the lower limbs, dysarthria, and respiration difficulty	Consistent
IV-10	Male	40	14	Hoarseness and swallowing difficulty	Dysarthria, swallowing difficulty, fasciculation of the tongue, and general weakness	Consistent
IV-12	Male	23	11	Hoarseness and swallowing difficulty	Dysarthria, respiration difficulty	Consistent
IV-19	Male	20	24	Weakness of the right lower limb	Dysarthria, dyspnea, weakness and atrophy in the both lower extremities, fasciculation in the left deltoid, and hyperactive DTR	Consistent
IV-20	Female	36	13	Weakness of the left lower limb	Reduced strength and fasciculation of the left lower limb, and decreased DTR in the left lower limb	Consistent
IV-21	Male	31	12	Weakness and atrophy of the lower limbs	Reduced strength and atrophy of the lower limbs, and dysarthria and respiration difficulty	NA
IV-23	Male	28	7	Weakness of the lower limbs	Fasciculation of tongue, hyperactive DTR, and no Babinski sign	Consistent

* Based on descriptions of the patient's uncle (III-1)

DTR; deep tendon reflexes, EMG; electromyography, NA; no available medical record

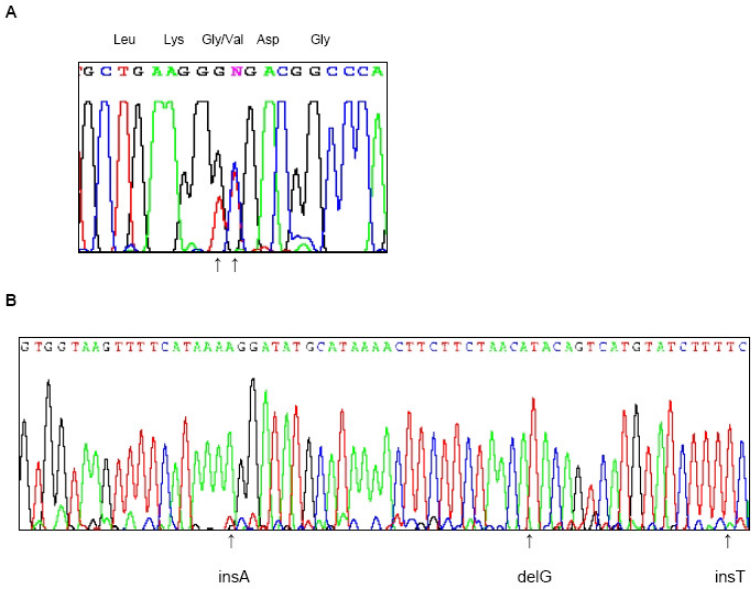

Figure 2. Automated sequence analysis of exon 1 and intron 4 from the *SOD1* gene showing the heterozygous G10V mutation and intronic alterations, respectively. The panel shows a GGC-to-GTT transition (arrow) that resulted in the substitution of valine for glycine (A) and non-amino-acid-altering variations [IVS4+15_16insA; IVS4+42delG; IVS4+59_60insT] in intron 4 (B). Green lines; adenine residues (A), blue lines; cytosine (C), black lines; guanine (G), red lines; thymine (T).

intronic alterations alone were found in the proband's brother (IV-8).

DISCUSSION

In the present study, we identified exonic mutation and intronic alterations in the *SOD1* gene in a Korean family with FALS. Patients with these mutations have differing clinical onset symptoms and anticipation (i.e., acceleration of the age at onset in successive generations).

The subpopulation of this family reported on previously, who had only G10V mutations, showed mostly lower motor neuron involvement, ascending progression, and rapid disease progression.[15] However, as a whole, *SOD1* mutations in this family resulted in different phenotypes, although anticipation was a unique feature, especially in the males. The observed anticipation may have been influenced by observer bias given that (1) the deduction of clinical onset in generation II based on anamnestic data can cause overinterpretation and (2) other (younger) family members who were not affected at the time of this study may develop the disease later. Mutation testing in all unaffected family members is necessary to completely resolve this issue, but ethical issues make this difficult. Further follow-up is warranted in at least one younger sister (IV-7) in the family who had a mutation of the *SOD1* gene and was alive without any symptoms.

Anticipation has been reported in several FALS families,[17-19] but its molecular basis remains speculative. It has been suggested that anticipation can result from additional genetic effects or from environmental factors.[4] An amino acid substitution at position 84 in Japanese and Italian families was found to be associated with generational anticipation, which might also have been associated with several factors such as being male and exposure to toxicants.[17,18] The presence of a G93S mutation in another Japanese family suggests that ethnicity influences this genetic mutation.[19] The G10V mutation was reported to occur exclusively in Korea, whereas other synonymous glycine[10] mutations were found in other ethnic groups.[12] Therefore, factors that generate anticipation in our family may be related to an ethnic–genetic interaction in addition to the effect of being male, although the role of the particular codon mutation itself cannot be excluded.

In our family, the pathogenic role of the intronic variations in the *SOD1* gene remains unclear. With few exceptions, mutations in the *SOD1* gene destabilize the structure of the SOD1 protein through metal-ion loss or perturbations of protein folding, and these destabilized SOD1 proteins either aggregate or turn over more

rapidly relative to the wild-type enzyme.[20] The proband and his elder sister (carrying mutations in the exons and introns of the *SOD1* gene) showed clinical symptoms that differed from those of previously reported family members, especially at the initial stage of the disease.[15] One possible explanation for the phenotypic differences and anticipation is intronic changes to the G10V mutation having an cumulative effect over generations. It is also possible that the intronic alterations of the *SOD1* gene contribute to the manifestations of FALS in other ways, such as by altering the pattern of gene expression by disturbing RNA splicing, or causing conformation changes of the gene product. However, since we did not functionally analyze the SOD1 enzyme activity or SOD1 at the protein level, further investigations are needed to elucidate the role of the intronic variations. In addition, it cannot be ruled out that these intronic variations represent a simple single-nucleotide polymorphism in the Korean population. Additional studies in a larger sample are required for confirmation.

In summary, we have described a family with FALS that showed autosomal-dominant inheritance and two distinct genetic alterations in Cu/Zn-*SOD1*, in which the affected family members exhibited different phenotypes and anticipation.

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