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Updated Genetic Analysis of Japanese Familial ALS Patients Carrying *SOD1* Variants Revealed Phenotypic Differences for Common Variants

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Abstract

Background and Objectives

Amyotrophic lateral sclerosis (ALS) is an adult-onset progressive neurodegenerative disease. Approximately 10% of ALS cases are familial, and more than 20 causative genes have been identified. As we have previously reported, *SOD1* variants are the most common causes of familial ALS in Japan. Because antisense oligonucleotides for *SOD1*-linked ALS are being used in practical applications, the types of variants and the clinical features of patients need to be updated.

Methods

We consecutively recruited 160 families with familial ALS in Japan. We performed genetic analyses, focusing on *SOD1*-linked ALS as the most common in our cohort, updated their genotypes, and characterized clinical phenotypes.

Results

A total of 26 *SOD1* variants in 56 patients and 49 families (30.6%) were collected, with the 3 most common (p.His47Arg [the conventional numbering; H46R], p.Leu127Ser [L126S], p.Asn87Ser [N86S]) accounting for 38.8% of all families. We also identified 2 novel variants (p.Ile36Phe [I35F] and p.Asn132Argfs*3 [N131Rfs*3]). The mean age at onset was 48.9 ± 12.2 (mean ± SD) years for all patients with *SOD1*-linked ALS. Lower limb onset comprised 70% of cases. The mean disease duration was 64.7 ± 82 months, and the median survival was 71.5 months. Some variants led to a relatively homogeneous phenotype, although clinical characteristics differed among types of variants and families. Patients with p.His47Arg (H46R) showed slower progression with lower limb onset and a predominance of lower motor neuron involvement. The p.Leu127Ser (L126S) variant led to varying degrees of progression in heterozygous or homozygous states and presented incomplete penetrance. Intrafamilial phenotypic differences were observed in families carrying p.Asn87Ser (N86S). Four variants (p.Cys7Gly [C6G], p.His44Arg [H43R], p.Leu85Val [L84V], and p.Cys147Arg [C146R]) were found to be associated with rapid disease progression.

Discussion

The genetic basis of familial ALS, at least for *SOD1* variants, still differed by geographic and ethnic background. Understanding these clinical profiles will help optimize evaluation in targeted gene therapy worldwide and benefit efficient diagnosis, leading to precise application in clinical practice.

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Glossary

ACMG = American College of Medical Genomics; **ALS** = amyotrophic lateral sclerosis; **HGMD** = Human Gene Mutation Database; **MND** = motor neuron disease; **NGS** = next-generation sequencing; **SOD1** = superoxide dismutase 1; **VUS** = variants of uncertain significance.

Introduction

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease characterized by selective degeneration of motor neurons. Its progressive clinical course with no cure is associated with a mean survival duration of 3–5 years.[1] Approximately 10% of ALS cases are classified as familial. In 1993, superoxide dismutase 1 (*SOD1*) gene variants were recognized as a cause of ALS.[2-4] Since then, more than 200 variants have been reported across all 5 exons of *SOD1*, most of which are missense variants (Figure 1). These *SOD1* variants accounted for approximately 12% of European familial cases of ALS, similar to the GGGGCC-hexanucleotide repeat expansion of *C9orf72*. In Asian populations, including Japan, Korea, and China, the most common variant is *SOD1* (25.1–35.1%) and patients with *C9orf72* repeat expansion are rare.[5] Thus, the pathogenic variant in *SOD1* is globally the most common cause of ALS.

SOD1 variants are mainly inherited as an autosomal dominant pattern with high or reduced penetrance but also a recessive pattern, including compound heterozygosity, or as a de novo variant. The NM_000454.5:c.272A>C, p.Asp91Ala (the conventional numbering; D90A) variant detected in the Scandinavian populations presents a recessive pattern and the highest allele frequency in Europe (0.01186 in Finnish European, 0.0007431 in non-Finnish European, and 0.000 in East Asian of the Genome Aggregation Database (gnomAD, gnomad.broadinstitute.org/). The second most common variant c.14C>T, p.Ala5Val (A4V) accounts for almost 36.4%–50% of *SOD1*-linked familial ALS cases in the United States.[6-8] These variants have not been reported in Japan, suggesting that some pathogenic variants are unevenly distributed among populations and are responsible for demographic features (Figure 1).

Patients with ALS carrying *SOD1* variants generally have clinical features with spinal onset from asymmetric lower limbs and predominantly lower motor neuron involvement (ALSoD; alsod.ac.uk),[7] although clinical phenotypes differ among carriers of the different *SOD1* variants. For example, patients with ALS carrying p.Asp91Ala (D90A) homozygous variant represent similar characteristics with slow involvement in some, including ataxia, bladder disturbance, and heat sensation.[7] p.His47Arg (H46R) and p.Gly42Asp (G41D) are known to lead to slower progression, with the patient surviving for a decade or more.[8,9] By contrast, patients carrying p.Ala5Val (A4V) have aggressive disease.[6] Furthermore, atypical extramotor features have been reported in some *SOD1* cases, such as cerebellar ataxia (p.Glu101Lys [E100K]),[10] sensory involvement (p.Leu9Val [L8V]),[11] autonomic failure (p.Cys147Arg [C146R]),[12] and multiple system dysfunction (p.Val32Ala [V31A]).[13] Mild cognitive affection has been found in *SOD1*-linked cases.[14,15] The patients with p.Gly42Ser (G41S), p.Ile114Thr (I113T), p.Leu145Phe (L144F), and p.Gly142* (G141*) presenting cognitive impairment resembling frontotemporal dementia have been also reported.[7,16-19]

Since 1991, our group has performed genetic analysis and clarified the genetic background in patients with familial ALS in Japan.[9] Subsequently, we described the genotypes and clinical features of 4 cases with *SOD1* variants.[11,20-22] We also recently reported that *SOD1* variants are the most common cause (32%; 36/111 families), with a frequency that is similar to that in other Asian populations.[23-25] With the recent breakthrough in the development of gene-specific treatment for patients with *SOD1*-related ALS, given the approval of the antisense oligonucleotide drug tofersen and other therapeutic strategies of the conjugate of short interfering RNA being in clinical trials, there is a significant need to unravel population-specific genotypes and phenotypes in familial ALS cases. In this study, we genetically analyze 160 families, including newly collected samples, focusing on *SOD1*-linked ALS as the most common in our cohort and update their genotype and clinical features. This extensive cohort study of Japanese *SOD1*-linked ALS provides accurate knowledge of possible regional differences among *SOD1* variants.

Methods

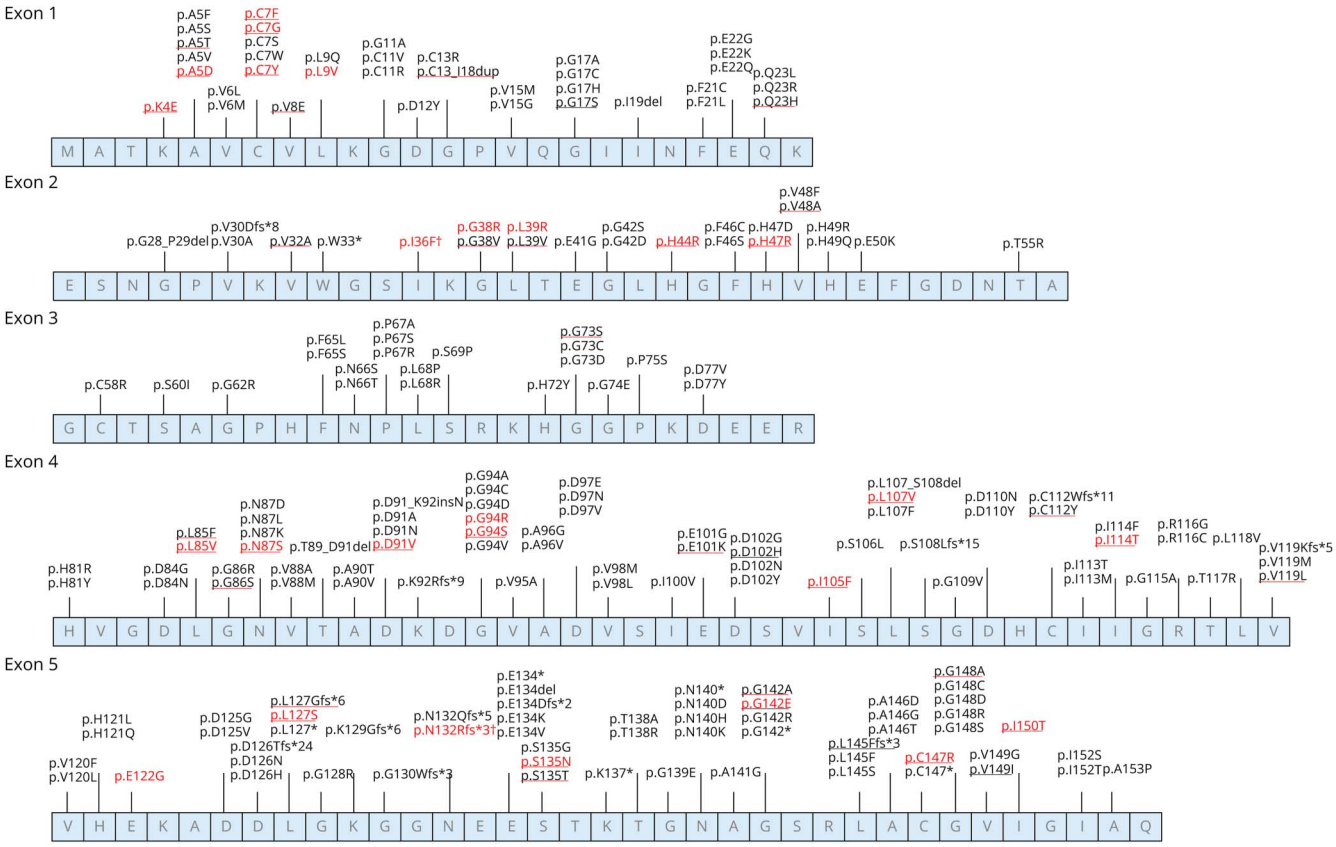
Standard Protocol Approvals, Registrations, and Patient Consents

All protocols were approved by the Ethics Committee of the Tohoku University School of Medicine (2021-1-022), and all patients gave their informed consent before genetic analysis.

Study Cohort

We recruited 160 families with familial ALS, as outlined by the revised El Escorial criteria from 1991 to 2022 at the Department of Neurology, Tohoku University Hospital, Japan. All patients from various regions throughout Japan had a positive familial history of ALS, motor neuron diseases (MNDs), or similar symptoms. To identify the genetic causes of Japanese familial ALS, we performed genetic analysis of samples by Sanger sequencing, single-strand conformational polymorphism of the products of PCR (PCR-SSCP), and/or next-generation sequencing (NGS), as previously described.[22-24] For these families carrying *SOD1* variants, we have already described *SOD1* variants as the most common cause (32%; 36/111 families) and the genotypes and clinical features of 4 cases.[11,20-22]

Figure 1 The Variants in *SOD1* for ALS



The predicted amino acid sequence of SOD1 (NP_000445.1) is shown. There are 211 variants and 6 intronic variants (c.72+19G>A, c.239+62T>C, c.358-304C>G, c.358-11A>G, c.358-10T>G, c.357+43_357+46delTACA) with DM of the variant class for ALS, MNDs, or SMA in HGMD 2022.3. Except for synonymous substitutions, data for *SOD1*-related variants in the ALSoD were collated and included if applicable to DM. *SOD1* (NM_000454.5) variants colored red were identified in our cohort. We identified 2 novel variants†. Variants with an underline have been identified in Japan (the red line shows variants identified in Japanese patients). ALS = amyotrophic lateral sclerosis; ALSoD = Amyotrophic Lateral Sclerosis online Database; DM = disease-causing mutation; HGMD = Human Gene Mutation Database; MNDs = motor neuron diseases; SMA = spinal muscular atrophy.

Genetic Analysis

DNA extraction from peripheral blood samples or a liver specimen from an autopsy case was performed using standard protocols. We performed whole-genome amplification using an Illustra GenomiPhi V2 DNA Amplification Kit (GE HealthCare, Vienna, Austria) for 1 sample before preparing libraries. To discover other pathogenic variants in addition to *SOD1*, we examined NGS analysis of genes associated with ALS or other MNDs using a previously described protocol.[24] We analyzed the screening panel of 35 targeted genes in 30 cases, the renewal panel of 63 expanded genes in 5 cases, and whole-exome sequencing (WES) in 9 cases (eTables 1 and 2). For WES, exon capture was performed with SureSelect Human All Exon Ver 6 Kit (Agilent Technologies, Santa Clara, CA), a capture-based approach involving sonication-based fragmentation. Libraries were sequenced using HiSeq 2500 (Illumina, San Diego, CA). Burrows-Wheeler Aligner was used to align the sequence reads to the hg19 human genome. After the removal of duplicates from the alignments, identification of single-nucleotide variants and indel calling were performed with Genome Analysis Toolkit.

Variant Analysis

Variants detected by NGS were annotated using ANNOVAR against the RefSeq and single-nucleotide polymorphism databases (ncbi.nlm.nih.gov/snp/). We extracted variants according to the applied filtering strategy.[23] Population genomic variations and minor allele frequencies were also referred in the Genome Aggregation Database (gnomAD, gnomad.broadinstitute.org/). In addition, we searched for known variants, including *SOD1*, listed as causative variants of ALS in the Human Gene Mutation Database (HGMD, provided by BIOBASE, Waltham, MA). We used prediction tools, including Polyphen2,[26] SIFT,[27] PROVEAN,[28] and Mutation Taster,[29] to assess the functional effects of variants. The pathogenicity assessment of variants was based on the American College of Medical Genomics (ACMG) classification standard. We describe the variant with Human Genome Variation Society nomenclature verified using the Mutalyzer program (mutalyzer.nl/). The conventional numbering of *SOD1* variants is shown in parenthesis to be consistent with previous articles.

Table 1 *SOD1* Variants Identified in Familial ALS

		Nucleotide change	Amino acid change		gnomAD v2.1.1		HGVD	jMorp				
Genomic location GRCh37	Exon	NM_000454.5	NP_000445.1	dbSNP156	global_AF	EAS_AF	AF	AF	PROVEAN	SIFT	PolyPhen-2	MutationTaster
chr21:33032092	1	c.10A>G	p.Lys4Glu (K3E)[b]	rs1568807297					Deleterious	Damaging	Probably damaging	Deleterious
chr21:33032096	1	c.14C>A	p.Ala5Asp (A4D)						Deleterious	Damaging	Probably damaging	Deleterious
chr21:33032101	1	c.19T>G	p.Cys7Gly (C6G)	rs1312702973	0.000003992	0			Deleterious	Damaging	Probably damaging	Deleterious
chr21:33032102	1	c.20G>A	p.Cys7Tyr (C6Y)	rs121912448	0.00003185	0			Deleterious	Damaging	Probably damaging	Deleterious
chr21:33032102-33032103	1	c.20_21delinsTT	p.Cys7Phe (C6F)[b]						Deleterious	Damaging	Probably damaging	Deleterious
chr21:33032107	1	c.25C>G	p.Leu9Val (L8V)[b]	rs1568807333					Neutral	Damaging	Possibly damaging	Deleterious
chr21:33036136	2	c.106A>T	p.Ile36Phe (I35F)[a]	rs1057524474					Deleterious	Damaging	Probably damaging	Deleterious
chr21:33036142	2	c.112G>A	p.Gly38Arg (G37R)	rs121912431					Deleterious	Damaging	Probably damaging	Deleterious
chr21:33036146	2	c.116T>G	p.Leu39Arg (L38R)	rs1555836520					Deleterious	Damaging	Probably damaging	Deleterious
chr21:33036161	2	c.131A>G	p.His44Arg (H43R)	rs121912435	0.000003976	0			Deleterious	Tolerated	Probably damaging	Deleterious
chr21:33036170	2	c.140A>G	p.His47Arg (H46R)	rs121912443					Deleterious	Damaging	Probably damaging	Deleterious
chr21:33039584	4	c.253T>G	p.Leu85Val (L84V)	rs121912452					Deleterious	Damaging	Possibly damaging	Deleterious
chr21:33039591	4	c.260A>G	p.Asn87Ser (N86S)	rs11556620				0.0001	Deleterious	Damaging	Probably damaging	Deleterious
chr21:33039603	4	c.272A>T	p.Asp91Val (D90V)	rs80265967					Deleterious	Damaging	Benign	Deleterious
chr21:33039611	4	c.280G>C	p.Gly94Arg (G93R)	rs121912437					Deleterious	Damaging	Probably damaging	Deleterious
chr21:33039611	4	c.280G>A	p.Gly94Ser (G93S)	rs121912437					Deleterious	Damaging	Possibly damaging	Deleterious
chr21:33039644	4	c.313A>T	p.Ile105Phe (I104F)	rs121912445					Deleterious	Damaging	Probably damaging	Deleterious
chr21:33039650	4	c.319C>G	p.Leu107Val (L106V)	rs121912440					Deleterious	Damaging	Probably damaging	Deleterious
chr21:33039672	4	c.341T>C	p.Ile114Thr (I113T)	rs121912441	0.00004772	0			Deleterious	Damaging	Probably damaging	Deleterious
chr21:33040791	5	c.365A>G	p.Glu122Gly (E121G)	rs1555836922					Deleterious	Tolerated	Probably damaging	Deleterious
chr21:33040806	5	c.380T>C	p.Leu127Ser (L126S)[b]	rs121912454					Deleterious	Damaging	Probably damaging	Deleterious
chr21:33040817-33040820	5	c.391_394dup	p.Asn132Argfs*3 (N131Rfs*3)[a]						NA	NA	NA	Deleterious
chr21:33040830	5	c.404G>A	p.Ser135Asn (S134N)	rs121912451	0.000003979	0			Deleterious	Damaging	Probably damaging	Deleterious
chr21:33040851	5	c.425G>A	p.Gly142Glu (G141E)	rs1568811489					Deleterious	Damaging	Probably damaging	Deleterious
chr21:33040865	5	c.439T>C	p.Cys147Arg (C146R)	rs1568811515					Deleterious	Damaging	Probably damaging	Deleterious

Continued

Table 1 *SOD1* Variants Identified in Familial ALS *(continued)*

Genomic location GRCh37	Exon	Nucleotide change	Amino acid change	dbSNP156	gnomAD v2.1.1		HGVD	jMorp	PROVEAN	SIFT	PolyPhen-2	MutationTaster
		NM_000454.5	NP_000445.1		global_AF	EAS_AF	AF	AF				
chr21:33040875	5	c.449T>C	p.Ile150Thr (I149T)	rs1424014997	0.000007959	0			Deleterious	Damaging	Probably damaging	Deleterious

Abbreviations: AF, allele frequency; EAS, East Asian.
[a] Novel variant.
[b] We describe the genotypes and clinical features of 4 cases with *SOD1*.[11,20-22]

Clinical Data Profile

Clinical data were collected from individual patients with confirmed *SOD1* variants and analyzed. In each case, the following clinical information was reviewed during genetic analysis or follow-up: age at onset, sex, initial symptom, clinical course, time of tracheostomy, and permanent ventilation (if applicable). When assessing the time of onset and duration of the disease, we made our best effort to identify the midpoint of each seasonal or annual period to ensure accuracy. In our cohort, we defined cases with a clinical course of less than 24 months as rapid and 36 months or longer as long progression unless otherwise specified. We conducted a *t* test between groups as needed.

Screening of Hexanucleotide Repeat Expansion in *C9orf72*

We screened all available samples except for 2 for the presence of the GGGGCC hexanucleotide expansion in the noncoding region of *C9orf72* through repeat primed PCR, as previously reported.[23,24] In brief, PCR was performed using a fluorescently labeled forward primer. The anchor primer, corresponding to the anchor tail of the reverse primer, included 7-deaza-2-deoxy-GTP. Capillary electrophoresis was performed using an ABI Prism 3130xl genetic analyzer or 3730xl DNA analyzer (Applied Biosystems, Foster City, CA). The data were analyzed using GeneMapper version 4.0 (Applied Biosystems) or Peak Scanner Software v2.0 (Applied Biosystems).

Data Availability

The data and materials are available from the corresponding authors on reasonable request.

Results

Genetic Analysis of the *SOD1* Gene

As a result of the analysis of 160 families with ALS, we identified 26 different variants in 49 families (56 cases), accounting for 30.6%, including 36 previously described[24] and 13 newly collected families (Table 1). Variants were identified across all exons, except the third exon. In variant databases, including gnomAD, Exome Aggregation Consortium (ExAC), and Japanese genetic variation databases, Human Genetic Variation Database (HGVD, genome.med.kyoto-u.ac.jp/SnpDB/) and jMorp (jmorp.megabank.tohoku.ac.jp/), each variant has rather low allele frequencies or it is not present, indicating that it is not a common benign variant in global populations. Based on in silico analysis in several databases, all variants are expected to be damaging. We identified 2 novel variants that were not registered in either HGMD or ALSoD: c.106A>T, p.Ile36Phe (I35F) and c.391_394dup, p.Asn132Argfs*3 (N131Rfs*3). The interpretation of these variants is likely pathogenic according to ACMG guidelines. None of the previously reported near-splice *SOD1* variants detectable from targeted NGS were detected.[24,30]

Known Variants in ALS-Related Genes

In screening for ALS-related genes using targeted NGS to discover oligogenic variant combinations of other pathogenic variants, known variants of uncertain significance (VUS) were

detected in 2 cases. A heterozygous variant of uncertain significance, c.6284T>C, p.Leu2095Ser, in *SPG11* (NM_0025137.4)[31] was detected in a patient carrying *SOD1* p.Leu85Val (L84V). Another heterozygous variant of uncertain significance, c.2212G>A, p.Glu738Lys, in *GARS1* (NM_002047.4)[24] was detected in a case involving p.Gly38Arg (G37R) in *SOD1*. In addition, the heterozygous c.505T>A, p.Trp169Arg variant in *SIGMAR1* (NM_005866.4) was identified in a patient with *SOD1* p.His47Arg (H46R).[32] Among 9 patients who underwent WES, we found the heterozygous c.308C>T, p.Pro103Leu variant in *DAO* (NM_001917.5) in a case involving p.Leu127Ser (L126S), which is a rare variant suggested to be a possible modifier of disease course in ALS.[33] No other known pathogenic ALS-related variant was identified. The hexanucleotide repeat expansion of *C9orf72* was not detected in available families with *SOD1* variants.

Clinical Characteristics of the Patients With *SOD1*

In our cohort, the p.His47Arg (H46R) and p.Leu127Ser (L126S) variants were the most common, together accounting for 30.6% of cases. The p.Asn87Ser (N86S) variant was the second most frequent. The mean age at onset was 48.9 ± 12.2 years. The age at onset ranged between variants, with the youngest carrying p.Ile36Phe (I35F) and the oldest carrying p.Ala5Asp (A4D) (Table 2, Figure 2A). Lower extremity onset accounted for 70.0% of cases. There were only 4 cases of the facial-onset or bulbar-onset type (7.1%), 3 of which had rapid progression with a duration of less than 2 years. Disease duration also varied among variant types, ranging from rapid progression with p.Cys7Gly (C6G) (3 months) to more than 200 months. The mean disease duration was 64.7 ± 82 months, with a median of 71.5 months (Table 2, Figure 2B).

There is considerable clinical variance among variants. Cases related to p.His47Arg (H46R) showed a prolonged course of lower limb onset without remarkable upper motor neuron signs. Cases involving p.Leu127Ser (L126S) demonstrated rapid progression of upper limb onset in a homozygous state but a long duration over a decade in the heterozygous state. 1 family had a dominant form with incomplete penetrance, in which the carrier, confirmed by genetic analysis, died of non-ALS causes without remarkable symptoms in his 80s. Families with p.Asn87Ser (N86S) also presented reduced penetrance. Clinical characteristics within the same family, including unaffected obligate carriers, were not homogeneous. Variants associated with rapid progression with an overall duration of less than 24 months in common among affected patients in the families that can be identified were found to be p.Cys7Gly (C6G), p.His44Arg (H43R), p.Leu85Val (L84V), and p.Cys147Arg (C146R). Patients with p.His44Arg (H43R) also exhibited atypical *SOD1*-linked phenotypes of frontotemporal lobe atrophy, as revealed by imaging after tracheostomy. Sensory neuropathy was found only in L8V cases.[11] In our cohort, none of the cases slowly progressing presented the urgency of the micturition of sphincter affection. No apparent cerebellar, extrapyramidal, or autonomic dysfunction was identified before tracheostomy or permanent ventilation.

Discussion

In this study, we describe updated genotypes and the clinical characteristics of *SOD1*-linked families. Presentations differed from the other populations and revealed their specific phenotype. Together with our previous findings,[23-25,34] we elucidated, to date, genetic contributions in 49.4% of 160 Japanese families in ALS (eFigure 1). *SOD1* variants were most common, and 26 variants across all exons, except for exon 3, in 49 families were detected (30.6%) in our cohort. The frequency of *SOD1* variants was relatively consistent with that of a previous report in Asian countries, ranging from 25.1% to 35.1%.[5] Among *SOD1*-linked families, the 3 most common variants (p.His47Arg [H46R], p.Leu127Ser [L126S], and p.Asn87Ser [N86S]) accounted for 38.8%. We did not identify patients with p.Ala5Val (A4V), the most common in North American populations, or p.Asp91Ala (D90A), with a high prevalence in Europe. Moreover, we identified only 1 patient harboring p.Ile114Thr (I113T), the third most common variant.[7] Our literature review of genetic analysis of Japanese familial ALS in 2 other groups also supports that p.His47Arg (H46R) is most common, followed by p.Leu127Ser (L126S), among the Japanese population (Table 3).[35,36] Altogether, the frequencies of variant types still differed among populations, distinct from those in Europe and the United States.[6,7]

Among the *SOD1* variants we identified in Japan, 2 novel variants, p.Ile36Phe (I35F) and p.Asn132Argfs*3 (N131Rfs*3), were absent in various population databases, including ExAC, gnomAD, HGVD, and jMorp. Amino acid 36 is highly conserved in vertebrates; the missense p.Ile36Phe (I35F) variant probably causes functional dysfunction according to deleterious predictions. The 4-bp duplicate variant (p.Asn132Argfs*3 [N131Rfs*3]) is predicted to cause a frameshift and prematurely terminate the protein at a highly conserved position. In silico analysis suggested deleterious effects that might cause nonsense-mediated mRNA decay (Table 1). SOD1 consists of homodimers, with 1 copper and 1 zinc ion bound to each subunit and 4 cysteines. The truncated protein has an open active site channel, increasing the catalysis of the reaction by simultaneously increasing the accessibility and flexibility of the metal ions and decreasing the specificity of metal ions, leading to harmful structural changes.[37] Furthermore, a frameshift and truncating variant in the same position (p.N132Qfs*5) has been reported for other patients with familial ALS.[38] Altogether, the pathogenicity of these variants is highly suspected despite the lack of cosegregation analysis.

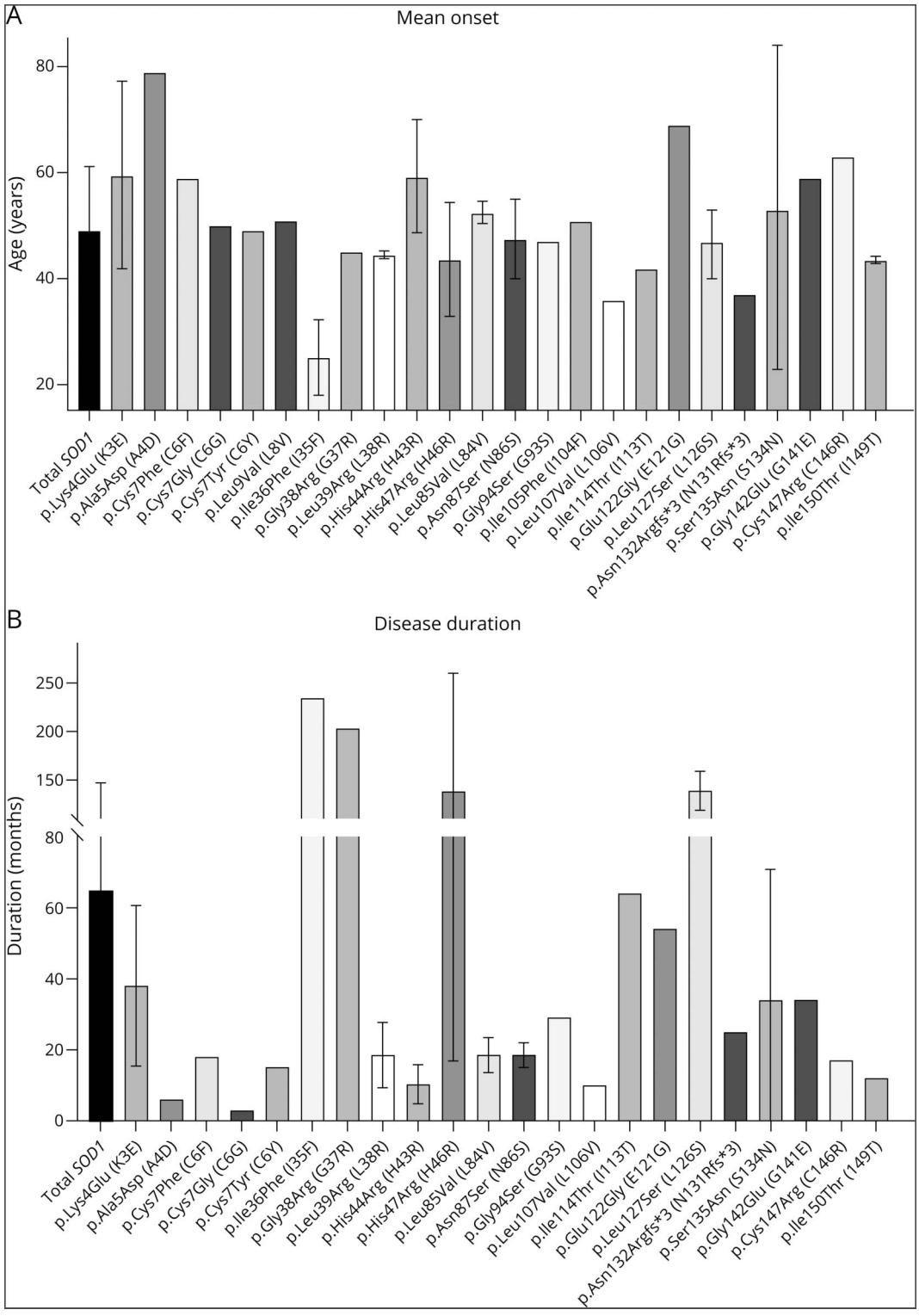
Although the age at onset in *SOD1*-linked patients widely ranges from 20 to 79 years, the mean of 48.9 years is comparably close to previous reports.[6,7,39] The disease duration varies from 3 months to more than 200 months. Patients with

Table 2 Disease Characteristics by Variant Type in *SOD1*-Linked Familial ALS

Amino acid change (NP_000445.1)	Number of families (%)	Number of patients	Mean age at onset, year ± SD (n)	Site of onset (n)	Mean duration, months ± SD (n)
p.His47Arg (H46R)	8 (16.3)	12	43.5 ± 10.8 (8)	Lower limb (8)	138.3 ± 121.4 (8)[a]
p.Leu127Ser (L126S)[b]	7 (14.3)	8	46.9 ± 5.9 (8)	Lower limb (6), upper limb (2)	138.8 ± 20 (4)[a]
p.Asn87Ser (N86S)	4 (8.2)	4	47.5 ± 7.5 (4)	Lower limb (4)	18.5 ± 3.5 (4)[a]
p.His44Arg (H43R)	3 (6.1)	4	59.3 ± 10.7 (4)	Lower limb (1), upper limb (2), limb (1)	10.3 ± 5.5 (4)[a]
p.Ile36Phe (I35F)	2 (4.1)	2	25 ± 7.1 (2)	Upper limb (1), lower limb (1)	234 (1)[a]
p.Leu39Arg (L38R)	2 (4.1)	2	44.5 ± 0.7 (2)	Upper limb (2)	18.5 ± 9.2 (2)
p.Leu85Val (L84V)	2 (4.1)	2	52.5 ± 2.1 (2)	Upper limb (1), face/upper limb (1)	18.5 ± 4.9 (2)
p.Ser135Asn (S134N)	2 (4.1)	2	53 ± 31.1 (2)	Lower limb (2)	34 ± 36.8 (2)[a]
p.Ile150Thr (I149T)	2 (4.1)	2	43.5 ± 0.7 (2)	Bulbar (1), lower limb (1)	12 (1)[a]
p.Lys4Glu (K3E)[b]	1 (2.0)	2	59.5 ± 17.7 (2)	Lower limb (1), face (1)	38 ± 22.6 (2)
p.Ala5Asp (A4D)	1 (2.0)	1	79 (1)	Upper limb	6 (1)[a]
p.Cys7Phe (C6F)[b]	1 (2.0)	1	59 (1)	Limb	18 (1)
p.Cys7Gly (C6G)	1 (2.0)	1	50 (1)	Lower limb	3 (1)
p.Cys7Tyr (C6Y)	1 (2.0)	1	49 (1)	Lower limb	15 (1)[a]
p.Leu9Val (L8V)[b]	1 (2.0)	1	51 (1)	Lower limb	Alive (1)
p.Gly38Arg (G37R)	1 (2.0)	1	45 (1)	Lower limb	203 (1)
p.Asp91Val (D90V)	1 (2.0)	1	NA	NA	NA
p.Gly94Arg (G93R)	1 (2.0)	1	NA	NA	NA
p.Gly94Ser (G93S)	1 (2.0)	1	47 (1)	Lower limb	29 (1)[a]
p.Ile105Phe (I104F)	1 (2.0)	1	51 (1)	Lower limb	Alive (1)
p.Leu107Val (L106V)	1 (2.0)	1	36 (1)	Lower limb	10 (1)[a]
p.Ile114Thr (I113T)	1 (2.0)	1	42 (1)	Lower limb	64 (1)
p.Glu122Gly (E121G)	1 (2.0)	1	69 (1)	Lower limb	54 (1)
p.Asn132Argfs*3 (N131Rfs*3)	1 (2.0)	1	37 (1)	Lower limb	25 (1)
p.Gly142Glu (G141E)	1 (2.0)	1	59 (1)	Lower limb	34 (1)
p.Cys147Arg (C146R)	1 (2.0)	1	63 (1)	Bulbar	17 (1)
	Total 49 families	Total 56	Total mean age at onset 48.9 ± 12.2 (50)	Lower limb (35/50)	Total mean disease duration 64.7 ± 82 (42)
		Male-female ratio 1 : 1.3		Upper limb (9/50)	Total median survival 71.5 (42)
				Limb (2/50)	
				Face/upper limb (2/50)	
				Bulbar (2/50)	

[a] The data included samples for which clinical information was not available from the last follow-up. Disease duration was calculated from onset to the time of last follow-up.
[b] We describe the genotypes and clinical features of 4 cases with *SOD1*.[11,20-22]

Figure 2 Mean Age at Onset and Disease Duration of Patients With *SOD1*-Linked ALS



(A) Mean age at onset of patients with *SOD1*-linked ALS. The average age at onset varied among various variants, with the youngest carrying p.Ile36Phe (I35F) and the oldest carrying p.Ala5Asp (A4D). Error bars represent SD for multiple samples. (B) The mean disease duration was collected for patients with *SOD1*-linked ALS. Disease duration varied among variant types, ranging from rapid progression in p.Cys7Gly (C6G) (3 months) to more than 200 months.

p.His47Arg (H46R) and p.Leu127Ser (L126S) present a long duration. Because p.His47Arg (H46R) cases included samples of which clinical information was not available from the last follow-up, the available data involved shorter duration than previously reported.[40] However, the lower limb onset, predominance of lower motor neuron involvement, and lack

Table 3 Review of Genetic Analysis of Japanese Familial ALS

Amino acid change	Number of Japanese familial patients				
NP_000445.1	Naruse et al. 2018	Nakamura et al. 2016	Our cohort	Total	%
p.Lys4Glu (K3E)	2/68		1/160	3/267	1.1
p.Ala5Asp (A4D)	1/68		1/160	2/267	0.7
p.Cys7Phe (C6F)			1/160	1/267	0.4
p.Cys7Gly (C6G)	1/68		1/160	2/267	0.7
p.Cys7Tyr (C6Y)			1/160	1/267	0.4
p.Leu9Val (L8V)			1/160	1/267	0.4
p.Ile36Phe (I35F)			2/160	2/267	0.7
p.Gly38Arg (G37R)			1/160	1/267	0.4
p.Leu39Arg (L38R)			2/160	2/267	0.7
p.Leu39Val (L38V)		1/39		1/267	0.4
p.His44Arg (H43R)	1/68		3/160	4/267	1.5
p.His47Arg (H46R)	3/68	3/39	8/160	14/267	5.2
p.Val48Ala (V47A)	1/68			1/267	0.4
p.Leu85Val (L84V)	1/68		2/160	3/267	1.1
p.Leu85Phe (L84F)	1/68			1/267	0.4
p.Asn87Ser (N86S)			4/160	4/267	1.5
p.Asp91Val (D90V)	1/68		1/160	2/267	0.7
p.Gly94Arg (G93R)			1/160	1/267	0.4
p.Gly94Ser (G93S)	3/68	2/39	1/160	6/267	2.2
p.Glu101Lys (E100K)	1/68			1/267	0.4
p.Ile105Phe (I104F)			1/160	1/267	0.4
p.Leu107Val (L106V)	2/68	3/39	1/160	6/267	2.2
p.Ile114Thr (I113T)	1/68		1/160	2/267	0.7
p.Val119Leu (V118L)	2/68			2/267	0.7
p.Glu122Gly (E121G)			1/160	1/267	0.4
p.Leu127Glyfs*6	1/68			1/267	0.4
p.Leu127Ser (L126S)	1/68	3/39	7/160	11/267	4.1
p.Asn132Argfs*3 (N131Rfs*3)			1/160	1/267	0.4
p.Ser135Asn (S134N)	1/68		2/160	3/267	1.1
p.Gly142Ala (G141A)		1/39		1/267	0.4
p.Gly142Glu (G141E)			1/160	1/267	0.4
p.Cys147Arg (C146R)	1/68		1/160	2/267	0.7
p.Gly148Ala (G147A)	1/68			1/267	0.4
p.Ile150Thr (I149T)	1/68	1/39	2/160	4/267	1.5
Total *SOD1*	27/68	14/39	49/160	90/267	33.7

of early symptoms in upper limbs and bulbar function were clearly represented. Patients with p.Ile36Phe (I35F) and p.Gly38Arg (G37R) may also have a long disease duration and milder progression rate, despite the small number of cases. Patients with more than 36 months of duration were characterized by lower limb onset, except for p.Ile36Phe (I35F) carriers, suggesting that screening the *SOD1* gene is important for patients showing these clinical features to help predict their prognosis. The 4 variants were associated with rapid progression in fewer than 24 months without intrafamilial variability. p.Cys7Gly (C6G), p.His44Arg (H43R), and p.Leu85Val (L84V) have been associated with rapid disease progression in previous reports, regardless of ethnic background.[39,41,42] Overall, contributing factors for the variability of phenotypes have yet to be determined. There was no correlation between disease severity and dismutation activity. Some variants associated with shorter disease duration are located toward the N-terminus of the protein, which suggests an association with the amyloid core of SOD1 fibrils.[7,39] Variants of conserved residues of SOD1 were significantly associated with shorter survival time in patients with ALS.[43] One of the possible factors for the phenotypic diversity of *SOD1*-linked ALS might be the location of the variant and the amino acid residue substituted, causing dimer instability and disruption of essential interactions in the SOD1 fibril structure.[44] Most families presented an autosomal dominant inheritance pattern; however, only 1 patient showed inheritance of the p.Leu127Ser (L126S) variant as a recessive trait under consanguinity. Some family members carrying p.Leu127Ser (L126S) or p.Asn87Ser (N86S) presented incomplete penetrance. The p.Leu127Ser (L126S) variant was also reported as one of the most common in Japanese patients with sporadic ALS (11/469), which may suggest the presence of incomplete disease penetrance.[35] Asymptomatic carriers have also been reported for several cases involving p.Asp91Ala (D90A), p.Ile113Met (I112M), p.Ile114Thr (I113T), p.Asn140His (N139H), and p.Gly141Ala (G141A).[45-48] The triggering factors contributing to the onset of the disease are still unknown, but no other known pathologic variants were detected in this study. Such heterogeneity among the families suggests other acquired environmental factors and further unsolved polygenic or oligogenic factors.

Because mild cognitive affection has been found in *SOD1*-linked ALS cases, to our knowledge, no cognitive impairment has been reported in patients with p.His44Arg (H43R). Frontotemporal atrophy based on imaging has been observed after early motor symptoms. Differences after the application of optional treatment, such as tracheostomy, may reveal their late symptoms. Recently, behavioral involvement has been reported to be more common than previously believed among patients harboring *SOD1* variants.[15] Behavioral assessment beginning at an early stage is needed.

The development of antisense oligonucleotide therapy that targets *SOD1*-linked ALS has begun, with hope based on the results of a reduction in neurofilament light chain level, the biomarker of axonal injury and neurodegeneration, but lacking significant differences according to ALS Functional Rating Scale-Revised.[49] Possible phenotypic confirmation by variants is still necessary to adequately assess treatment efficacy. Especially for patients with variants outside clinical trials, it may help to determine effectiveness and optimize treatment. We should consider that low penetrance exists when precisely judging the effectiveness of early treatment interventions.

There are some limitations to this study. (1) Clinic-based studies are potentially affected by referral bias. A mild female predominance (M:F ratio = 1:1.3) was present in our cohort. However, the findings are supported by a previous report,[50] and there were no significant differences in age at onset and disease duration by sex and no female predominance in the bulbar-onset type. (2) There were limitations of accessible clinical data for the censored cases. There was no detailed information about therapeutic interventions, such as riluzole or edaravone. (3) Owing to a lack of availability, we could not conduct a comprehensive genetic analysis for some samples. In addition, neurofilament in CSF or blood could not be analyzed. Additional data must be collected in future prospective large cohorts.

Despite these limitations, this study revealed certain clinical attributes in specific variant types in *SOD1* and that the frequency of variant types differs among different populations. The data presented herein provide a practical perspective on intervention timing and appropriate applicants for *SOD1*-linked ALS. This genetic profile may contribute to optimizing treatment efficacy and selectivity in the future.

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