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Oligogenic basis of sporadic ALS

The example of *SOD1* p.Ala90Val mutation

Liina Kuuluvainen, MD, Karri Kaivola, MD, Saana Mönkäre, MSc, Hannu Laaksovirta, MD, Manu Jokela, MD, PhD, Bjarne Udd, MD, PhD, Miko Valori, MSc, Petra Pasanen, PhD, Anders Paetau, MD, PhD, Bryan J. Traynor, MD, PhD, David J. Stone, PhD, Johanna Schleutker, PhD, Minna Pöyhönen, MD, PhD, Pentti J. Tienari, MD, PhD,* and Liisa Myllykangas, MD, PhD*

Correspondence
Dr. Myllykangas
email.liisa.myllykangas@helsinki.fi

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Abstract

Objective

To characterize the clinical and neuropathologic features of patients with amyotrophic lateral sclerosis (ALS) with the superoxide dismutase 1 (*SOD1*) p.Ala90Val mutation, as well as the mutation frequency and the role of oligogenic mechanisms in disease penetrance.

Methods

An index patient with autopsy-proven ALS was discovered to have the *SOD1* p.Ala90Val mutation, which was screened in 2 Finnish ALS cohorts (n = 453). Additional contributing variants were analyzed from whole-genome or whole-exome sequencing data.

Results

Seven screened patients (1.5%) were found to carry the *SOD1* heterozygous mutation. Allele-sharing analysis suggested a common founder haplotype. Common clinical features included limb-onset, long disease course, and sensory symptoms. No TDP43 pathology was observed. All cases were apparently sporadic, and pedigree analysis demonstrated that the mutation has reduced penetrance. Analysis of other contributing genes revealed a unique set of additional variants in each patient. These included previously described rare *ANG* and *SPG11* mutations. One patient was compound heterozygous for *SOD1* p.Ala90Val and p.Asp91Ala.

Conclusions

Our data suggest that the penetrance of *SOD1* p.Ala90Val is modulated by other genes and indicates highly individual oligogenic basis of apparently sporadic ALS. Additional genetic variants likely contributing to disease penetrance were very heterogeneous, even among Finnish patients carrying the *SOD1* founder mutation.

*These authors contributed equally to this work.

From the Department of Clinical Genetics (L.K.), Helsinki University Hospital; Department of Medical Genetics (L.K.), University of Helsinki, Helsinki, Finland; Molecular Neurology (K.K., M.V., P.J.T.), Research Programs Unit, Biomedicum, University of Helsinki, Helsinki, Finland; Department of Medical Genetics (S.M.), University of Helsinki, Helsinki, Finland and Turku; University Hospital (S.M.), Laboratory Division, Genetics and Saske, Department of Medical Genetics, Turku, Finland; Department of Neurology (H.L.), Helsinki University Hospital, and Molecular Neurology, Research Programs Unit, Biomedicum, University of Helsinki, Helsinki, Finland; Neuromuscular Research Center (M.J., B.U.), Tampere University Hospital and University of Tampere, Tampere, Finland; Division of Clinical Neurosciences (M.J.), Turku University Hospital and University of Turku, Turku, Finland; Folkhälsan Research Center (B.U.), Biomedicum, University of Helsinki, Helsinki, Finland; Institute of Biomedicine (P.P., J.S.), University of Turku; Turku University Hospital (P.P., J.S.), Laboratory Division, Genetics and Saske, Department of Medical Genetics, Turku, Finland; Department of Pathology (A.P.), University of Helsinki and Helsinki University Hospital, Helsinki, Finland; Laboratory of Neurogenetics (B.J.T.), National Institute on Aging, National Institutes of Health, Bethesda, MD; Merck & Co. (D.J.S.), Inc., West Point, PA; Department of Clinical Genetics (M.P.), Helsinki University Hospital; Department of Medical Genetics (M.P.), University of Helsinki, Helsinki, Finland; Department of Neurology (P.J.T.), Helsinki University Hospital; and Department of Pathology (L.M.), University of Helsinki and Helsinki University Hospital, Helsinki, Finland.

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Glossary

ALS = amyotrophic lateral sclerosis; **IHC** = immunohistochemistry; ***SOD1*** = superoxide dismutase 1; **WES** = whole-exome sequencing; **WGS** = whole-genome sequencing.

Superoxide dismutase 1 (*SOD1*) mutations are the second most common cause of familial amyotrophic lateral sclerosis (ALS) explaining approximately 12%–20% of familial and 1%–2% of sporadic ALS.[1] Usually, *SOD1* mutations have an autosomal dominant pattern of inheritance.[1]

SOD1 mutation c.269C>T, p.Ala90Val (previously called A89V) has been described in 3 family members with ALS with variable age at onset, incomplete penetrance, and sensory neuropathy[2] and in 4 additional individuals with ALS.[3–5] The ethnicity of the patients was not reported.

We identified the *SOD1* p.Ala90Val mutation through whole-exome sequencing (WES) in our neuropathologically examined index patient with ALS and investigated its frequency and additional genetic burden in 2 Finnish ALS cohorts.

Methods

The index patient was autopsied because of a clinically atypical motor neuron disease. DNA was extracted from his liver tissue, and a heterozygous *SOD1* p.Ala90Val mutation was found in WES performed at the Institute for Molecular Medicine Finland (FIMM, Helsinki, Finland). This mutation was screened in 2 ALS cohorts. The Helsinki cohort (n = 300), collected 1995–2014, was subjected to whole-genome sequencing (WGS) at Broad Institute, Boston, MA. The Turku cohort (n = 153) consisted of samples sent to the TYKS Laboratory of Medical Genetics between 2007 and 2016 for *SOD1* sequencing with the diagnosis of definitive or probable ALS or phenotype consistent with motor neuron disease in the referral. WES was performed at FIMM to the p.Ala90Val mutation–positive samples of the Turku cohort. Sequencing details are shown in e-Methods. All p.Ala90Val-positive samples were screened for the *C9orf72* repeat expansion using the previously described method.[6]

To identify additional coding or splicing variants in the p.Ala90Val-positive samples, we analyzed other neurodegenerative disease and *SOD1* pathway genes from their WES/WGS data (e-methods and table e-1, links.lww.com/NXG/A152).

Neuropathologic analysis was performed following the standard protocol. Clinical information was examined from medical records.

Standard protocol approvals, registrations, and patient consents

This study was approved by the local ethics committees. Informed consent was given by the patients/relatives, or the approval for the use of patient tissue samples was obtained from the National Supervisory Authority for Welfare and Health (Valvira).

Data availability statement

The data set is available upon reasonable request from the corresponding author.

Results

Genetic analyses

The *SOD1* mutation NM_000454.4 c.269C>T, p.Ala90Val found in the index patient was analyzed in the Helsinki and Turku cohorts (n = 453). Seven additional heterozygous cases were found (1.5%). This mutation is in the gnomAD database[7] in 1/8,367 Finnish samples (heterozygote) but absent in all other populations (95,693 samples) after removing neurologic patients. There is a statistically significant difference in the carrier frequency of the p.Ala90Val between the Finnish patients with ALS (7/453, excluding index) and the Finnish gnomAD population (1/8,367) ($p = 6.9 \times 10^{-9}$, Fisher exact test).

Although the patients were not known to be related, allele-sharing analysis of the samples indicates a common haplotype of at least 379,7 kb (Chr21:32723906-33103636) with 8 rare single nucleotide polymorphism markers, implying a common ancestor (table e-2, links.lww.com/NXG/A152).

None of the patients had a family history of ALS, and altogether 6 unaffected carriers (aged 50–87 years) of p.Ala90Val were identified in the families of P6 and P8 (figure e-1, links.lww.com/NXG/A152). Analysis of other neurodegeneration implicated genes (n = 1,115) revealed that all patients had additional potentially contributing variants (table and table e-3, links.lww.com/NXG/A152). Each patient had a unique profile of other variants, the number of possibly or probably contributing variants varied between 4 and 14 per patient. Seven of the 8 patients had at least 1 variant that we considered "probably pathogenic" (table and table e-3, links.lww.com/NXG/A152). Three patients had mutations previously described in ALS: P6, a heterozygous *ANG* mutation; P7, a heterozygous *SPG11* mutation; and P8 was compound heterozygous for *SOD1* p.Ala90Val and p.Asp91Ala confirmed by family member testing (figure e-1, links.lww.com/NXG/A152). Four other patients had probably pathogenic variants in genes previously associated with motor neuron disease or peripheral neuropathy: P1 in *ARHGEF28*, P3 in *UNC13A*, P4 in *ARHGEF10*, and P5 in *ADGRB2/BAI2*. P2 was the only one who did not have any probably pathogenic

Table Clinical features and selected genetic findings of patients 1–8

	P1	P2	P3	P4	P5	P6	P7	P8
Patient	**Male**	**Female**	**Female**	**Male**	**Female**	**Female**	**Male**	**Female**
Age at onset (y)	40	51	70	47	43	32	48	50
Disease duration (y)	14	7	7	18	25[a]	6[a]	15[a]	7
Site of onset	Lower limb	Limb[b]	Upper limb	Lower limb	Lower limb	Lower limb	Lower limb	Lower limb
Initial symptoms	Cramps, difficulties with balance, and diminished control of legs	NA	Weakness of limbs, predominantly right upper limb	Difficulty walking, stumbling, and problems with balance	Muscle twitches	Distal lower limb weakness	Pain and later weakness in the lower limbs	Distal lower limb weakness
Sensory symptoms	Yes	NA	No	Yes	Yes	No	Yes	No
Initial EMG	Sensorimotor polyneuropathy	NA	Motor axon damage, suggestive of motor neuron disease	Consistent with motor neuron disease	Consistent with motor neuron disease	Compatible with motor neuron disease	Compatible with motor neuron disease	Compatible with motor neuron disease
Sensory neuropathy in EMG	Yes[c]	NA	No	Yes[c]	No	No	No	No
Cognitive symptoms	No	NA	Yes[d]	No	No	No	No	No
Cerebral infarct in MRI	No	NA	Yes[d]	Yes	No	NA	NA	NA
Creatine kinase	Elevated	NA	Normal	Elevated	Slightly elevated	Normal		Normal
Cause of death	ALS	Suspected myocardial infarction	ALS	Respiratory failure	[a]	[a]	[a]	ALS
Family history of ALS	No	NA	No	No	No	No	No	No
C9orf72	Normal	Normal	Normal	Normal	Normal	Intermediate allele (23 repeats)	Normal	Normal
Probably pathogenic variants in WES/WGS/ other tests	WES: *ARHGEF28*: p.T248R	WGS[e]	WGS: *UNC13A:* p.R298W	WGS: *ARHGEF10*: p.P234T	WGS: *ADGRB2/ BAI2*p.S63L	WES: *ANG:* p.K78E[f]	WES: *SPG11:* p.Q1875X *CACNA1H:* p.R1231C homozygous *SMN2* deletion[g]	WES: *1.SOD1:* p.D91A[f]

Abbreviations: ALS = amyotrophic lateral sclerosis; NA: information not available; WES = whole-exome sequencing; WGS = whole-genome sequencing.
[a] The patient is alive.
[b] More detailed information about the site of onset is not available.
[c] The amplitude of antidromic sensory potentials of patient P1 at age 40 years: median nerve 4.8 mV (normal value ³20 mV), ulnar nerve 3.6 mv (normal value ³17 mV), and sural nerve 5.8 mV (normal value ³6 mV), and of patient P4 at age 53 years: ulnar nerve 4.9 mV and sural nerve 8.7 mV; the median nerve had no response in the study. The EMG studies were performed using the standard protocol.
[d] In addition to small old infarcts in the left occipital lobe and right posterior frontal area, there was a mild expansion in the cortical liquor spaces and mild atrophy in the hippocampi, changes in the pons area and in the periventricular white matter that were interpreted as degenerative. This patient also had cognitive symptoms, and a neuropsychological assessment at age 76 years revealed predominantly frontal lobe problems that were not at the level of dementia.
[e] The patient had variants in 3 *SOD1* pathway genes: *FBXW8*, *NOB1*, and *ALOX15*.
[f] Mutation has been previously reported in patients with ALS; the references are in the supplemental material (e-references, links.lww.com/NXG/A152).
[g] Deletions in exons 7 and 8 of the *SMN1* and *SMN2* genes were investigated by the PCR-restriction fragment length polymorphism method. Comprehensive list and information of genetic variants are in table e-3, links.lww.com/NXG/A152.

variants according to our interpretation; she had nevertheless variants in 3 *SOD1* pathway genes: *FBXW8*, *NOB1*, and *ALOX15* (table e-3, links.lww.com/NXG/A152). None of the patients had a *C9orf72* repeat expansion, but P6 had 23 hexanucleotide repeats in *C9orf72* (the significance of which is presently unclear).

Clinical features

The patients' clinical features are summarized in the table. The age at onset was variable (32–70 years). All had a limb-onset disease, with typical presenting symptoms including fasciculations, weakness, and difficulties with walking and balance. The initial EMG and nerve conduction study of P1 (index) revealed sensorimotor polyneuropathy; later, he had stocking-like sensory abnormalities in both feet, and both soles showed hyperesthesia in addition to the motor symptoms. The initial EMG of P4 was consistent with motor neuron disease, and a later EMG revealed additional distal sensory polyneuropathy. P7 had reduced vibration sense in his feet, and P5 had paresthesia in her hands. All patients had a long disease course, 7–25+ years; 3 of the patients were still alive at the time of this study.

Neuropathologic features

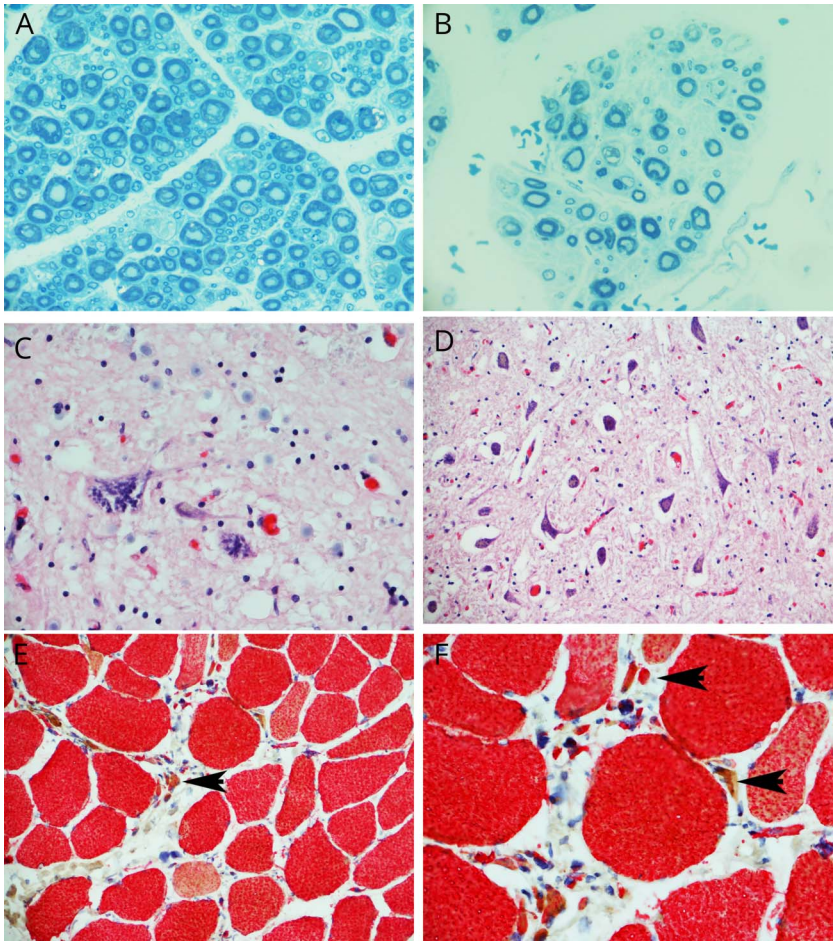
The index patient's brain weighed 1527 g and appeared macroscopically normal. The anterior roots of the spinal cord were atrophic. Microscopically, the anterior horns showed significant loss of neurons (figure, C).

The axon density was markedly lowered in the anterior roots compared with the dorsal roots (figure, A–B).

There was mild neurodegeneration in the hypoglossal nucleus at the level of the medulla oblongata (figure, D). Immunohistochemistry (IHC) showed no TDP43-positive inclusions in the anterior horns, cortical areas, or in the hypoglossal nucleus. No hyaline conglomerate inclusions, reported to be specific for some *SOD1* mutations,[8] were detected on neurofilament (SMI32) IHC. Tau, and beta amyloid stainings were negative.

P62 staining showed only a few positive neurites, but no intraneuronal inclusions. The muscle samples showed very strong group atrophy and fairly abundant reinnervation (figure, E–F). The cause of death was concluded to be motor neuron disease.

Figure Neuropathologic findings of the autopsied patient (index)



(A) Plastic-embedded sections from the dorsal spinal root show normal density of axons, whereas (B) severe loss of both myelinated and unmyelinated axons is seen in the anterior spinal roots (toluidine blue ×600 magnification). (C) There is severe neuronal loss in the anterior spinal columns, and the remaining neurons appear chromatolytic (hematoxylin and eosin [HE]-stained section from the lumbar spinal cord, ×400 magnification). (D) The hypoglossal nucleus was mildly degenerated (HE-stained section from the medulla oblongata, ×400 magnification. (E) Muscle biopsy taken from the vastus lateralis showed atrophic small groups (arrow) and overrepresentation of type 2 fibers, suggesting abundant reinnervation (double immunohistochemistry for myosin, ×200 magnification). (F) Higher magnification shows that both type 1 (brown) and type 2 (red) fibers (arrows) are atrophic (×400 magnification).

Discussion

In this study, 1.5% of the patients with ALS carried the *SOD1* mutation p.Ala90Val, making it a major mutation in Finnish patients with ALS based on its frequency, although it had previously been described in only 7 patients.[2–5] In the Helsinki cohort, it is the third most common currently known ALS mutation after *C9orf72* repeat expansion and *SOD1* p.Asp91Ala (unpublished data). There is a clear enrichment of p.Ala90Val in the Finnish population.

There were 6 unaffected family members who were confirmed to carry the p.Ala90Val mutation illustrating the proposed reduced penetrance and oligogenic mechanisms in ALS.[4] The *SOD1* p.Ala90Val probably plays a dominating role in our patients despite the additional rare variant burden because (1) the clinical features were similar in all patients thus far reported[2] and (2) the neuropathology of the index patient was consistent with *SOD1*-related ALS.[9] The p.Ala90Val mutation has been shown to cause a conformational change on the SOD1 protein,[10] and SOD1 enzymatic activity has been shown to be reduced in the CSF of a patient with the mutation.[5] In silico analysis with MutationTaster (mutationtaster.org/), PolyPhen-2 (genetics.bwh.harvard.edu/pph2/), and SIFT (provean.jcvi.org/index.php) predicts p.Ala90Val to be deleterious.

We cannot exclude the role of environmental factors in disease penetrance with total confidence. However, 3 of the 8 patients had mutations previously described in ALS, and 4 additional patients had probably pathogenic rare variants in genes previously implicated in motor neuron disease or peripheral neuropathy. Our data represent an illustrative example of a mutation whose penetrance appears to require additional genetic factors. It also demonstrates the genetic heterogeneity of sporadic ALS: despite sharing a founder mutation, the spectrum of other variants was very heterogeneous; each patient had a unique set of variants. The small sample size and varying sequencing methodology preclude powerful analyses of the discovered variants on clinical features. At present, it is not possible to make firm conclusions on the pathogenic role of the potentially contributing variants in individual patients, although in the p.Asp91Ala compound heterozygous P8, the disease-causing effect is clear. The allele frequency of many variants (table e-3, links.lww.com/NXG/A152) suggests predisposing or disease-modifying rather than disease-causing effects.

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Disclosure

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Appendix Authors

Name	Location	Role	Contribution
Liina Kuuluvainen, MD	University of Helsinki, Helsinki, Finland	Author	Design, analysis, and interpretation of data, WES and WGS analyses, and drafted and revised the manuscript critically for important intellectual content
Karri Kaivola, MD	University of Helsinki, Helsinki, Finland	Author	Design, analysis, and interpretation of data, WES and WGS analyses, and drafted and revised the manuscript critically for important intellectual content
Saana Mönkäre, MSc	University of Helsinki, Helsinki, Finland	Author	C9orf72 screening, Sanger sequencing and data collection, and drafted and revised the manuscript critically for important intellectual content
Hannu Laaksovirta, MD	University of Helsinki, Helsinki, Finland	Author	Clinical data and sample collection and drafted and revised the manuscript critically for important intellectual content
Manu Jokela, MD, PhD	University of Tampere, Tampere, Finland, and University of Turku, Turku, Finland	Author	Design, analysis, and interpretation of data, clinical data and sample collection, family member testing, and drafted and revised the manuscript critically for important intellectual content
Bjarne Udd, MD, PhD	University of Tampere, Tampere, Finland	Author	Design, analysis, and interpretation of data, clinical data and sample collection, family member testing, and drafted and revised the manuscript critically for important intellectual content

Continued

Appendix *(continued)*

Name	Location	Role	Contribution
Miko Valori, MSc	University of Helsinki, Helsinki, Finland	Author	WES and WGS analyses, selection of the SNP markers used in the allele-sharing analysis, and drafted and revised the manuscript critically for important intellectual content
Petra Pasanen, PhD	University of Turku, Turku, Finland	Author	Sanger sequencing and data collection and drafted and revised the manuscript critically for important intellectual content
Anders Paetau, MD, PhD	University of Helsinki, Helsinki, Finland	Author	Neuropathologic analysis and drafted and revised the manuscript critically for important intellectual content
Bryan J Traynor, MD, PhD	Laboratory of Neurogenetics, National Institute on Aging, NIH, Bethesda, MD	Author	Drafted and revised the manuscript critically for important intellectual content
David J Stone, PhD	Merck & co., Inc., West Point, PA	Author	Drafted and revised the manuscript critically for important intellectual content
Johanna Schleutker, PhD	University of Turku, Turku, Finland	Author	Clinical data and sample collection, drafted and revised the manuscript critically for important intellectual content
Minna Pöyhönen, MD, PhD	University of Helsinki, Helsinki, Finland	Author	Clinical data and sample collection and drafted and revised the manuscript critically for important intellectual content
Pentti J Tienari, MD, PhD	University of Helsinki, Helsinki, Finland	Author	Design, analysis, and interpretation of data, statistical analysis, and drafted and revised the manuscript critically for important intellectual content
Liisa Myllykangas, MD, PhD	University of Helsinki, Helsinki, Finland	Author	Design, analysis, and interpretation of data, neuropathologic analysis, and drafted and revised the manuscript critically for important intellectual content

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