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*  
Neurol Genet 2019;5:e335. doi:10.1212/NXG.0000000000000335

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.T.), Research Programs Unit, Biomedicum, University of Helsinki, Helsinki, Finland; Department of Medical Genetics (S.M.), University of Helsinki, Helsinki, Finland; Department of Pathology (A.P.), University of Helsinki and Helsinki University Hospital, Helsinki, Finland; Department of Neurology (B.U.), Tampere University Hospital and University of Tampere, Tampere, Finland; Division of Biomedical Research (B.U.), Biomedical Research Programs Unit, 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 Neurology (H.L.), Helsinki University Hospital, and Molecular Neurology Research Programs Unit, Biomedicum, University of Helsinki, Helsinki, Finland; National Institute on Aging, National Institutes of Health, Bethesda, MD; and Merck & Co. (D.J.S.), Inc., West Point, PA; Department of Medical Genetics (M.P.), University of Helsinki; Department of Clinical Genetics (L.M.), University of Helsinki and Helsinki University Hospital, Helsinki, Finland.

Funding information and disclosures are provided at the end of the article. Full disclosure form information provided by the authors is available with the full text of this article at Neurology.org/NG.

The Article Processing Charge was funded by the University of Helsinki.

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND), which permits downloading and sharing the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.
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 neuropathy2 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 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).

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 database7 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 mutation between the Finnish patients with ALS (7/453, excluding index) and the Finnish gnomAD population (1/8,367) (p = 6.9 x 10^-9, Fisher exact test).

Although the patients were not known to be related, allelesharing 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 ARHGGEF28, P3 in UNC13A, P4 in ARHGGEF10, and P5 in ADGRB2/BAI2. P2 was the only one who did not have any probably pathogenic

Glossary

ALS = amyotrophic lateral sclerosis; IHC = immunohistochemistry; SOD1 = superoxide dismutase 1; WES = whole-exome sequencing; WGS = whole-genome sequencing.
| Patient | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 |
|---------|----|----|----|----|----|----|----|----|
|         | 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<sup>a</sup> | 6<sup>a</sup> | 15<sup>a</sup> | 7 |
| Site of onset | Lower limb | Limb<sup>b</sup> | 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<sup>c</sup> | NA | No | Yes<sup>c</sup> | No | No | No | No |
| Cognitive symptoms | No | NA | Yes<sup>d</sup> | No | No | No | No | No |
| Cerebral infarct in MRI | No | NA | Yes<sup>d</sup> | Yes | No | NA | NA | NA |
| Creatine kinase | Elevated | NA | Normal | Elevated | Slightly elevated | Normal | Normal | Normal |
| Cause of death | ALS | Suspected myocardial infarction | ALS | Respiratory failure | a | a | a | ALS |
|家族史 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<sup>e</sup> | WGS: UNCI3A: p.R298W | WGS: ARHGEF10: p.P234T | WGS: ADGRB2/BAI2p.S63L | WES: ANG: p.K78E<sup>f</sup> | WES: SPG11: p.Q1875X | WGS: CACNA1H: p.R1231C homozygous SMN2 deletions<sup>g</sup> | WES: 1.SOD1: p.D91A<sup>i</sup> |

Abbreviations: ALS = amyotrophic lateral sclerosis; NA: information not available; WES = whole-exome sequencing; WGS = whole-genome sequencing.

<sup>a</sup> The patient is alive.

<sup>b</sup> More detailed information about the site of onset is not available.

<sup>c</sup> The amplitude of antidromic sensory potentials of patient P1 at age 40 years: median nerve 4.8 mV (normal value ≥20 mV), ulnar nerve 3.6 mV (normal value ≥17 mV), and sural nerve 5.8 mV (normal value ≥16 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.

<sup>d</sup> 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.

<sup>e</sup> The patient had variants in 3 SOD1 pathway genes: FBXW8, NOB1, and ALOX15.

<sup>f</sup> Mutation has been previously reported in patients with ALS; the references are in the supplemental material (e-references, links.lww.com/NXG/A152).

<sup>g</sup> 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.

<sup>i</sup> The patient had variants in 3 SOD1 pathway genes: FBXW8, NOB1, and ALOX15.
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, 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)
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 reported2 and (2) the neuropathology of the index patient was consistent with SOD1-related ALS.5 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.9 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.

Acknowledgment

The authors thank Lilja Jansson, Leena Saikko ja Kristiina Nokelainen for technical assistance in this study.

Study funding

This study was supported by Helsinki University Hospital, Sigrid Juselius Foundation, Finnish Cultural Foundation, the Academy of Finland (294817), Liv och Hälsa Foundation and Finska Läkaresällskapet, The Finnish Medical Foundation, and the Intramural Research Program of the National Institute on Aging, NIH (Z01-AG000949-02). The whole-genome sequencing was funded by Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, and Intramural Research Program of the NIH.

Disclosure

Disclosures available: Neurology.org/NG.

Publication history

Received by Neurology: Genetics January 7, 2019. Accepted in final form April 1, 2019.

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 Monkäre, 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
References

1. Marangi G, Traynor BJ. Genetic causes of amyotrophic lateral sclerosis: new genetic analysis methodologies entailing new opportunities and challenges. Brain Res 2015;1607:75–93.

2. Rezania K, Yan J, Dellefave L, et al. A rare Cu/Zn superoxide dismutase mutation causing familial amyotrophic lateral sclerosis with variable age of onset, incomplete penetrance and a sensory neuropathy. Amyotroph Lateral Scler Other Motor Neuron Disord 2003;4:162–166.

3. Andersen PM, Sims KB, Xin WW, et al. Sixteen novel mutations in the Cu/Zn superoxide dismutase gene in amyotrophic lateral sclerosis: a decade of discoveries, defects and disputes. Amyotroph Lateral Scler Other Motor Neuron Disord 2003;4:62–73.

4. Cady J, Alfred P, Bala T, et al. Amyotrophic lateral sclerosis onset is influenced by the burden of rare variants in known amyotrophic lateral sclerosis genes. Ann Neurol 2015;77:100–113.

5. Jacobsson J, Jonsson PA, Andersen PM, Forsgren L, Marklund SL. Superoxide dismutase in CSF from amyotrophic lateral sclerosis patients with and without CuZn-superoxide dismutase mutations. Brain 2001;124:1461–1466.

6. Renton AE, Majounie E, Waite A, et al. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron 2011;72:257–268.

7. Lek M, Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature 2016;536:285–291.

8. Ince PG, Tomkins J, Slade JY, Thatcher NM, Shaw PJ. Amyotrophic lateral sclerosis associated with genetic abnormalities in the gene encoding Cu/Zn superoxide dismutase: molecular pathology of five new cases, and comparison with previous reports and 73 sporadic cases of ALS. J Neuropathol Exp Neurol 1998;57:895–904.

9. Mackenzie IR, Bigio EH, Ince PG, et al. Pathological TDP-43 distinguishes sporadic amyotrophic lateral sclerosis from amyotrophic lateral sclerosis with SOD1 mutations. Ann Neurol 2007;61:427–434.

10. Fujisawa T, Homma K, Yamaguchi N, et al. A novel monoclonal antibody reveals a conformational alteration shared by amyotrophic lateral sclerosis-linked SOD1 mutants. Ann Neurol 2012;72:739–749.
Oligogenic basis of sporadic ALS: The example of SOD1 p.Ala90Val mutation
Liina Kuuluvainen, Karri Kaivola, Saana Mönkäre, et al.
Neurol Genet 2019;5;
DOI 10.1212/NXG.0000000000000335

This information is current as of April 23, 2019

Updated Information & Services
including high resolution figures, can be found at:
http://ng.neurology.org/content/5/3/e335.full.html

References
This article cites 10 articles, 0 of which you can access for free at:
http://ng.neurology.org/content/5/3/e335.full.html##ref-list-1

Citations
This article has been cited by 2 HighWire-hosted articles:
http://ng.neurology.org/content/5/3/e335.full.html##otherarticles

Subspecialty Collections
This article, along with others on similar topics, appears in the following collection(s):
All Clinical Neurology
http://ng.neurology.org/cgi/collection/all_clinical_neurology
All Genetics
http://ng.neurology.org/cgi/collection/all_genetics
Amyotrophic lateral sclerosis
http://ng.neurology.org/cgi/collection/amyotrophic_lateral_sclerosis_

Permissions & Licensing
Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at:
http://ng.neurology.org/misc/about.xhtml#permissions

Reprints
Information about ordering reprints can be found online:
http://ng.neurology.org/misc/addir.xhtml#reprintsus