The SOD1-mediated ALS phenotype shows a decoupling between age of symptom onset and disease duration

Superoxide dismutase (SOD1) gene variants may cause amyotrophic lateral sclerosis, some of which are associated with a distinct phenotype. Most studies assess limited variants or sample sizes. In this international, retrospective observational study, we compare phenotypic and demographic characteristics between people with SOD1-ALS and people with ALS and no recorded SOD1 variant. We investigate which variants are associated with age at symptom onset and time from onset to death or censoring using Cox proportional-hazards regression. The SOD1-ALS dataset reports age of onset for 1122 and disease duration for 883 people; the comparator population includes 10,214 and 9010 people respectively. Eight variants are associated with younger age of onset and distinct survival trajectories; a further eight associated with younger onset only and one with distinct survival only. Here we show that onset and survival are decoupled in SOD1-ALS. Future research should characterise rarer variants and molecular mechanisms causing the observed variability.

In 1993, variants in the gene superoxide dismutase 1 (SOD1, [NM_000454]) were identified as a causal factor in people with amyotrophic lateral sclerosis (ALS), through analysis of 13 different families with 11 different SOD1 missense mutations. SOD1 variants are reported in 15% of people with familial ALS in European populations, 30% of people with familial ALS in Asian populations, and 1–2% of people with apparently sporadic ALS in both populations. Limited information is available on other populations.

SOD1-mediated ALS is characterised by distinct features related to the clinical and pathological phenotype. Since the discovery that variants in SOD1 can cause ALS, over 180 variants have been identified and they are distributed throughout the gene and protein. This is in contrast to other genetic determinants of ALS, for example mutations in FUS, C9orf72 and TARDBP, where variants are concentrated in specific functional domains of the protein. In SOD1-mediated ALS there is very little reported association with cognitive impairment, which, depending on cut-offs for neuropsychological deficits is estimated to occur in up to 50% of people with sporadic ALS in population-based studies. People with SOD1-ALS are often reported to have a lower motor neuron predominant phenotype, with more frequent limb onset than is observed in typical ALS. At the cellular level, TDP-43 protein aggregates, which are the pathological hallmark in >95% of ALS cases, are absent in most people with SOD1-mediated ALS implying that a different mechanistic pathway leads to motor neuron death.

Within the SOD1 ALS population, certain variants are associated with atypical disease progression compared to ALS as reported in population-based studies. For example, the p.A5V variant is associated with shorter survival and the homozygous p.D91A variant with longer survival. Demographic factors also correlate with survival. For example, men with SOD1-mediated ALS have shorter survival than women. Other variants, such as p.D125V and p.H44R have been associated with faster disease progression in an Australian population. As gene-specific therapies for ALS are being developed it is important to understand the prognostic implications of specific variants. This was demonstrated in a trial of Tofersen, an anti-sense oligonucleotide targeting the knock down of SOD1 mRNA, where a significant impact on disease progression was noted in a subset of patients carrying the p.A5V variant, who typically have a rapid disease progression.
Some variants in SOD1 may be coincidentally found in people with ALS but not cause their disease. One way of assessing this is to compare age of symptom onset in people with SOD1 variants and in people with sporadic ALS. In the liability threshold model of disease, a disease which is consistent with ALS risk, if an individual’s liability passes a threshold, disease develops. According to the multistep model of ALS disease risk, people take on average 6 molecular steps to develop ALS, but people with SOD1 variants need on average 2 steps—interpreted as SOD1 variants accounting for 4 of the 6 steps. If ALS variants increase risk of disease, we should expect them to lower the age of onset, through increasing a person’s liability to disease from birth. To date most genotype-phenotype correlations in SOD1 mediated ALS are from case reports, single-centre clinic databases, and reviews. Here, we analysed the phenotypic and demographic characteristics of people with ALS with a known SOD1 variant in a large, international dataset, to define the impact of individual variants on the age of symptom onset and survival. Understanding which variants cause the disease and their effect on the phenotype will improve genetic counselling, interpretation and application of clinical trial results and understanding of pathological mechanism.

**Results**

**Case description**

Once data were cleaned there were 1383 SOD1-ALS cases, each with a non-synonymous variant for analysis, demographic and clinical characteristics are summarised in (Table 1). Almost all records (99%), had a recorded diagnosis of ALS. The remaining 1% were recognised ALS-variants progressive muscular atrophy or primary lateral sclerosis. As the comparator dataset contained 11% of people with a diagnosis of either primary lateral sclerosis or progressive muscular atrophy, which could affect median disease duration, we ran time-to-event analyses only on those people with a recorded diagnosis of ALS according to El Escorial criteria in the comparator dataset (including all categories Definite to Suspected) and ALS without further definition in the SOD1 dataset. There were 12,622 records in the comparator dataset. In both datasets, most people had limb onset ALS, but the proportions were quite different between the two, with 74% spinal onset in the SOD1-ALS dataset compared to 63% in the comparator dataset. Age of onset in the SOD1-ALS dataset was about 49 years compared to 61 years in the comparator dataset. Diagnostic delay was on average 10 months in people with SOD1-ALS compared 12 months for people with sporadic ALS, and survival from onset was ~28 months compared to 35 months.

Of the records in the SOD1-ALS dataset, 1122 had complete information needed to analyse the effect of the variant on age of onset and 833 had complete data needed to analyse disease duration; the equivalent numbers in the comparator dataset were 10,214 and 9101, respectively. For more details, including which records were excluded, please see the CONSORT diagram (Fig.1).

**Time-to-event analyses**

There was considerable variation in survival time and age of symptom onset by variant as shown in Fig. 2. The box plots include people whose survival has been right censored and are not adjusted for other factors such as site of onset. For this reason, we compared the time-to-event distributions of each individual variant where there were sufficient replicates to the population-based estimates using Cox proportional hazards regression. Variants with a p value smaller than the Bonferroni corrected p value for each Cox proportional hazards analysis and a sample size of 10 or more people per group (for each variable age of onset and disease duration) are shown in grey in Fig.2 and are summarised in the Forest plots Figs. 3 and 4. The remainder of the results for variants with sample sizes of 9 or less are in the source file.

Of the variants analysed with a sample size of 10 or more people, 16 variants were associated with age of onset, and 9 variants with disease duration. All strongly associated variants have a younger median age of onset than the comparator cohort. However, unlike age of onset, a third of the variants with different survival distributions had a longer survival than the typical ALS population, this is visualised in Fig. 4. Kaplan-Meier plots can be found in Supplementary Figs. S2 and S3. Of the nine variants that were associated with different survival to sporadic ALS, eight were also associated with developing the disease at a younger age. There is a lack of correlation between average age of onset of symptoms and log average disease duration as shown in Supplementary Fig. S4. All analyses of disease duration by variant included age of onset as a covariate.

**Table 1 | Demographic features of people with SOD1 ALS**

| Diagnosis | SOD1 dataset Total n = 1383 | Comparator dataset Total n = 12,622 | p value (comparison) |
|-----------|-----------------------------|-----------------------------------|---------------------|
| ALS (incl flail limb) | 1370 99 | 11333 89.8 | <0.001 (chi squared test) |
| PLS/PMA | 13 1 | 1134 8.9 | - |
| Not recorded | - | 155 1.2 | - |
| Site of onset | | | |
| Spinal | 1026 74.2 | 7957 63 | <0.001 (chi squared test) |
| Bulbar | 108 7.8 | 3518 27.8 | - |
| Mixed | 8 0.58 | - | - |
| Respiratory | 8 0.58 | - | - |
| Not recorded | 233 16.8 | 1147 9.1 | - |
| Mean age of onset years (SD) | 48.9 (12.8) NA 61.1 (12) NA <0.001 (t-test) |
| Gender Female: Male: Not recorded | 655: 726: 2 47.4: 52.5: 0.1 5137:7481:4 41:59:0.0003 <0.001 (chi squared test) |
| Family history Yes: No: Not recorded | 969: 185: 229 70.1: 13.4: 16.5 1853: 7016: 3753 14.3: 55.5: 28.7 <0.001 (chi squared test) |
| Median diagnostic delay months (IQR) | 10 (19.3) NA 12 (14) NA 0.003 (Mann-Whitney U) |
| Median disease duration months (IQR) | 27.7 (61.0) NA 35.1 (35.6) NA <0.001 (Mann-Whitney U) |
| Dead Yes: No: Unknown* | 861: 284: 238 62.3: 20.5: 17.2 9108: 1677: 1837 72.2: 13.2: 14.5 <0.001 (chi squared test) |

Of the records with unknown diagnosed status, 48 in the SOD1 dataset and 1 in the STRENGTH dataset had disease duration data available. Two-sided tests used to compare variables. Comparing median disease duration of people with deceased status recorded and those without this variable recorded in the SOD1 dataset showed no difference (Mann Whitney U test p value 0.22). These records and the comparator were excluded from the Cox proportional hazards models analysing disease duration.

ALS primary lateral sclerosis, PMA progressive muscular atrophy, SD standard deviation, IQR interquartile range.
**Fig. 1 | Modified CONSORT diagram of datasets included in analysis.** The diagram shows the number of records identified from the following sources: ALS online Database, Project MinE, ALS Clinic databases, STRENGTH and the US population dataset. Records were excluded for missing or spurious data, or because of the diagnostic phenotype.

**Fig. 2 | Box plots of age of symptom onset and disease duration by variant.** Information is displayed for those variants where there were >9 cases. The centre value is the median and boxes represent interquartile range with whiskers representing the minima and maxima values associated with each variant. A Box plot showing age of symptom onset by variant, n = 976. B Box plot of log survival by variant, n = 809. C Selected box plots of log survival faceted by codon to highlight differences and similarities in survival distribution, n = 415. Source data are provided as a Source Data file.
Variants represented in the Forest plots are visualised on SOD1 dimers and these are shown in Figs. 5 and 6.

Variants within the dimer interface are associated with shorter survival (HR 2.69 (95% CI 2.4, 3) p-value = 1.28 × 10^{-69} and variants in any functional domain are associated with younger age of onset when compared to the comparator dataset, which likely reflects that many SOD1 variants reduce age of onset.

**Discussion**

In this study we have analysed the clinical phenotype of a large international dataset of people with ALS who have known pathogenic variants in SOD1. Their presentation differs from previous reports of the population-based ALS cohorts and the cohort we used as a comparator population\(^27\). We observed a higher percentage of limb onset ALS at 74% compared to 63% from population-based ALS cohorts and the comparator cohort. The age of symptom onset and overall survival are lower than sporadic ALS, although this is not the case when p.A5V variants are excluded from the dataset—median disease duration is longer at 45 months. There is still a slight male preponderance of 1.1 compared to 1 if all variants conformed to a mendelian autosomal dominant pattern of inheritance. Given that SOD1 variants account for four of the six presumed steps taken to develop ALS (according to the multistep model of ALS in population-based cohorts and subsequent recalculations in genetic subtypes) the other steps may be related to risks that men are exposed to more than women\(^17\). Most people in the dataset have a family history of ALS but about 10% are listed as being apparently sporadic. De novo mutations in SOD1 are rare and the absence of a family history is more likely to be due to incomplete penetrance, inadequate record keeping or small family size, which may mask familiarity, supported by identity by descent analysis that has identified familial links in apparently sporadic SOD1 ALS\(^28-30\).

We have compared the age of symptom onset and survival in SOD1-ALS to a population-based comparator cohort to identify variants that statistically significantly affected the clinical phenotype (i.e. had p values lower than the respective multiple-testing thresholds of 0.0007 and 0.00082). Of the 16 variants (with group size >9) associated with age of onset, 8 were also associated with survival, some of which had a longer median survival. This apparent
The context of the multistep and liability threshold models of disease, sample sizes in these groups were
onset passed the multiple testing threshold p-value, although the
comparator dataset, and none of the four with an older median age of
onset had a younger median age of onset than people with ALS in the
neurodegenerative disorders. Htt are due to protein misfolding and may be true for other genetic
scenarios could be relevant to ALS as the toxicity of mutant SOD1 and
independent process that then leads to cell death. Either of these
variant damages cells, causing disease onset followed by a variant-
independent process that then leads to cell death. Either of these
scenarios could be relevant to ALS as the toxicity of mutant SOD1 and
Htt are due to protein misfolding and may be true for other genetic
neurodegenerative disorders.

Sixty-six of the 70 variants analysed for differences in age of
onset had a younger median age of onset than people with ALS in the
comparator dataset, and none of the four with an older median age of
onset passed the multiple testing threshold p-value, although the
sample sizes in these groups were five people or fewer. Interpreted in
the context of the multistep and liability threshold models of disease,
both of which are consistent with ALS risk, this trend, along with the
high number of variants passing the multiple-testing threshold
implies that the variants are likely to be risk variants rather than being
randomly found in people with ALS, although this study does not
replace other epidemiological methods. 70% of people in the SODI-
ALS dataset had a family history of ALS, however earlier recorded age
of onset is not likely to be due to ascertainment bias as people with
sporadic ALS with a high genetic liability have younger age of onset31.
In the SODI dataset there is no difference in age of onset between
people a positive family history and those with a negative family
history HR 1.01 (95% CI 0.83, 1.24), p value = 0.9. As people with a
SODI variant but with a negative family history can be considered
people with a high genetic liability, this supports those findings.
Inheritance patterns in ALS have been reported as Mendelian, poly-
genic and oligogenic and it is possible that variants in different parts
of the SODI gene cause risk through each of these various patterns34.
Variant in SODI could contribute to polygenic or oligogenic risk and
this may be related to differing TDP-43 pathology in some cases of
SODI-ALS.

In contrast to the frequent observation of a relationship between
SODI mutation and reduced age of onset, only a few variants differ-
entially influenced survival, and a third of those appeared to be
protective, lengthening rather than shortening survival. The variants
most strongly associated with shorter disease duration tended to be
closer to the N-terminus of the protein (at positions 5, 7, 21 and 42),
which may relate to the amyloid core of both wild-type and mutant
SOD1 fibrils being located towards the N-terminus of the protein but
there were exceptions35. A change from glycine to serine at codon 42
was associated with longer disease duration but a change from gly-
cine to aspartic acid was not, this is in stark contrast with variants at
codon 5, which were both associated with shorter survival. At codon
7, only C7G was associated with shorter disease duration but a change from glycine to aspartic acid was not, this is in stark contrast with variants at
codon 5, which were both associated with shorter survival. At codon
7, only C7G was associated with shorter disease duration despite all
three variants at codon 7 being associated with younger disease
onset, and similarly at codon 94 only G94A was associated with
shorter disease duration and not younger age of onset whereas G94C
and G94D were associated with younger age of onset and not disease
duration. There is likely an interaction between location, the nature
of the amino acid substitution and the location in the protein struc-
ture, and subsequent effect on the thermodynamic stability of the
SOD1 dimer, the creation of additional fibril-forming seed regions or
alteration of packing around the existing fibril cores, and other fac-
tors as yet undetermined. Solving this problem was beyond the scope
of this study, but we hope that the data presented here will aid in the
design of further experimental and in silico studies to identify such
complex correlations.

Understanding which factors cause disease and which affect
clinical progression will improve genetic counselling and development of
therapies. There are currently clinical trials using SOD1 antisense
oligonucleotides and the largest effect may be observed in the var-
iants with the shortest survival time.13 It is not clear what impact
therapy will have on people carrying variants associated with a longer
disease duration, although if effective, the therapy should halt pro-
gression altogether. However, for people with slow-progression var-
iants, the effect of gene therapy may be more difficult to prove without
lengthy observation. In trials of lithium in ALS, there was a survival
benefit in people with variants in the UNCL34 gene, and this brought
them in line with ALS survival in the control group without UNCL34
variants. Analysing subgroups based on faster progressing variants
may be appropriate, and useful for people before they receive therapy
to understand their survival benefit.

The limitations of this study are that it is mainly based on clinic
populations or single case studies and a large proportion of the dataset
is made up of people from the US with p.A5V variants which may not be
generalisable to the global SOD1-ALS population. This underscores the
need to characterise phenotype by variant in analyses. The clinical data
for people with SOD1-ALS are limited in scope and there is missing
data. In our survival modelling, the comparator population is
European-derived and does not represent a comparator dataset for all
of the countries represented in the SOD1 dataset. As a sensitivity
analysis we have run our main analysis, restricting the SOD1 dataset to
only those countries represented in the comparator dataset. Although
this reduces the number of variants available for analysis our conclu-
sions remain the same in that there are more variants associated with
younger age of onset than shorter survival and many of the same
variants have strong associations with both outcome measures. There
may be people represented in both datasets, although this is more
likely for UK and Italian people as there were not many people with
SOD1-ALS in Irish, Dutch and Belgian populations, and it is at most a
very small proportion of the comparator dataset. We plan to develop a
web-tool using the dataset from this study so people can use com-
parator populations they feel are appropriate when analysing their
own data.

We have characterised the effect of a number of SOD1 variants on
the ALS phenotype but, some SOD1 variants are very rare and a larger
number of ALS patients harbouring those variants is needed to study
them. Additional work is needed to characterise the molecular
mechanism behind this variability of effect on the clinical phenotype.

Methods
Data sources
The data analysed in this project were either in the public domain
(phenotype information sources from scientific publications) or were
fully anonymised at source and therefore completely anonymous at
the point of access. No new data were collected for this study. Fol-
lowing King’s College London Research Governance protocols ethical
clearance was not required for this study.

SOD1 cohort. We primarily accessed the ALS Online Database, a
manually curated collection of published evidence about genes and
genetic variants associated with ALS (https://alsod.ac.uk). The data-
base includes clinical data collected from individual or family case
reports of 150 genes including SOD1, with data available at variant
level. In the instance of missing data, corresponding authors were
contacted to ask for further information. We also contacted clinicians
working in specialist centres that performed genetic testing and
requested they provide anonymised records of people with SOD1-ALS.
Each data source and their local ethical approval are detailed below:

Macquerie University: participants recruited under informed
written consent as approved by the Human Research Ethics Commit-
tee of Macquerie University.

ANZAC Research Institute: participants recruited under informed
written consent as approved by the institutional review board of the
ANZAC Research Institute (Sydney South West Area Health Service).

University of Massachusetts: data were acquired with formal
patient consent according to protocols reviewed and approved by the
Institutional Review Boards of first the Massachusetts General Hospital
and then the University of Massachusetts Medical School.

University Hospitals of Montpellier: all participants consented for
storage of their data and its use in research, the study was approved by
the Ethics committee (CCPRB) of Pitié Salpêtrière Hospital n°31/92.

King’s College London: participants provided consent for storage
of their genetic and clinical data and its use for research in protocols
approved by Local Research Ethics Committee approval number
222/02.

Washington University School of Medicine in St Louis: the data
was collected under a waiver of consent since the participants were all
deceased.

Peking University Third Hospital: all patients included provided
written informed consent to participate in the clinical and genetic
studies, which were approved by the institutional ethics committee of
Peking University Third Hospital (PUTH).

Northwestern Medicine – Feinberg School of Medicine: North-
western’s Institutional Review Board has reviewed and approved our
Neurological Diseases Registry annually since 1991. Consents include
the statement that data obtained from studying the subject’s con-
tributions may be shared with other researchers as long as the data is
deidentified.

Istituto Auxologico Italiano IRCCS-University of Milan: data were
collected in the project SOD1-ITALS approved by Ethical Committee of
the IRCCS Istituto Auxologico Italiano.

University of Belgrade: all individuals gave written informed
consent for the storage of their data and its use in research and the
Ethics Committee of the School of Medicine at the University of Bel-
grade approved this protocol.

Koc University: data and sample collection was approved by
Bogaziçi University Ethics Committee. Signed informed consent was
obtained from all subjects. The storage of the data and its use for
research was approved by the patients.

Project MinE: the Project MinE database was searched for people
with ALS in whom SOD1 variants had been identified by whole genome
sequencing the ethical approval for the project MinE dataset is described in
detail elsewhere8.

Comparator cohort. To compare age of onset and survival in people
from the general ALS population and SOD1-mediated ALS we used a
comparator population of people from population-based datasets of
ALS in five European populations (UK, Netherlands, Italy, Ireland and
Belgium) and the United States. The data from European countries
consisted of clinical variables only that were originally collected and
analysed as part of the Survival, Trigger and Risk, Epigenetic, eNVi-
ronmental and Genetic Targets for motor neuron Health (STRENGTH)
project. The ethical approval for the European and US datasets are
described in detail elsewhere8,9,10.

Additionally, countries included in the study are visualised in
Supplementary Fig. S1.

Clinical and demographic variables–SOD1 dataset
People were eligible if they had a recorded diagnosis of ALS made by
a Consultant Neurologist, or their diagnosis was published as ALS in
the literature. Two people were described as having ALS-flail limb,
and these were coded as ALS. We collected sex at birth and age of
onset (in years) of first motor symptoms of ALS, defined as first weakness or speaking or swallowing difficulty. Site of onset was coded as bulbar, spinal, respiratory or mixed. We asked whether people had a family history of ALS as reported by their clinician with no specific definition. To record disease progression, we collected or requested the time in months from onset of motor symptoms to diagnosis as well as the months until death, or their most recent appointment date. Additionally, we asked whether the person was deceased or not as a binary variable, and where this was missing, we coded it as the person not being deceased. Finally, we asked whether the person had been diagnosed with dementia; this was not specified as being a formal diagnosis of frontotemporal dementia. As data were fully anonymised, we were not able to use personal identifiers to find duplicate records. Records from different sources but with the same variant, country of origin, gender, age of onset and site of onset were assumed to be duplicates.

Genetic variants
Amino acid change was denoted by genomic location (rather than the historic notation that not including the initial methionine, for example we used p.A5V/p.D91A rather than A4V/D90A). In some cases, this can lead to ambiguity. For example, position 113 and 114 in this format are both isoleucine, so that I113 could refer to I113 or I114 if the format is not specified. In such situations, the original source was checked for how it referred to other non-ambiguous variants, so for example if a case study source referred to someone as having an A4V variant and someone else as an I113T we assumed they were using the format not including the initial methionine recorded the ambiguous variant as p.I114T. Where it was not possible to determine which format the variant referred to, if the variant was impossible given the DNA sequence at that codon and it could not be clarified, or if the source referred to a general location, for example just recording an insertion into an exon with no further details, these were classed as ‘ambiguous’ and excluded. Additionally, we did not analyse data on synonymous variants. For readability ‘p.’ has been left off the graphs when referencing a variant.

Functional location of genetic variants
Amino acids that are within 6 Å of the dimer interface were classed as being within the dimer interface. The codons making up the electrostatic loop and dimer interface were defined according to those amino acids identified as being in those areas according to the literature. If the codon was in the dimer interface and the electrostatic loop or the zinc loop, they were classified in those locations rather than in the dimer interface.
The codon numbers and their corresponding location are:
Dimer Interface: 4–10, 18–20, 50–55, 60–62, 112–116, 148–154
Electrostatic loop: 122–134
Zinc loop: 51–84
Pymol version 1.7.1.1 was used to plot variants associated with age of onset and disease duration onto PDB structure 9c2v.

Statistical analysis
Frequencies of phenotype groups in the SOD1 dataset were compared with those in the population-based ALS comparator dataset using descriptive statistics.
Time-to-event analysis to assess which variants are strongly associated with age of onset and disease duration from onset was performed using Cox proportional hazards regression for variants found in three or more people. Due to the unreliability of Cox proportional hazards regression at smaller sample sizes only those with 10 or more cases are displayed in the main manuscript, the remainder can be found in the source data file. Models were adjusted for site of onset of symptoms and gender the coxph() function was utilised with tie resolution at the default setting. In addition we calculated Cohen’s $D$ for goodness of fit using the royston() function with default settings. When modelling disease duration from onset, age of onset was also included as a covariate. Modelling by functional location of variants was performed separately but the covariates were the same for each outcome.
There were 70 variants eligible for time-to-event analysis modelling for age of onset and 61 available for time-to-event analysis of survival; the Bonferroni corrected $p$-value thresholds for these analyses were 0.0007 and 0.00086, respectively. Data were analysed and visualised in R version 4.0.2 using the packages ‘ggplot2’ (version 3.3.5) ‘worldmap’ (version 1.3-6) and ‘survival’ (version 3.2-7).

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Additional information

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