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ORIGINAL COMMUNICATION

Predictors for progression in amyotrophic lateral sclerosis associated to SOD1 mutation: insight from two population-based registries

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Abstract

Background Uncovering distinct features and trajectories of amyotrophic lateral sclerosis (ALS) associated with *SOD1* mutations (*SOD1*-ALS) can provide valuable insights for patient' counseling and stratification for trials, and interventions timing. Our study aims to pinpoint distinct clinical characteristics of *SOD1*-ALS by delving into genotype–phenotype correlations and factors that potentially impact disease progression.

Methods This is a retrospective observational study of a *SOD1*-ALS cohort from two Italian registers situated in the regions of Emilia-Romagna, Piedmont and Valle d'Aosta.

Results Out of 2204 genotyped ALS patients, 2.5% carried *SOD1* mutations, with a M:F ratio of 0.83. *SOD1*-ALS patients were younger, and more frequently reported a family history of ALS and/or FTD. *SOD1*-ALS had a longer survival compared to patients without ALS-associated gene mutations. However, here was considerable variability in survival across distinct SOD1 mutations, with an average survival of less than a year for the L39V, G42S, G73S, D91N mutations. Among *SOD1*-ALS, multivariate analysis showed that, alongside established clinical prognostic factors such as advanced age at onset and high progression rate at diagnosis, mutations located in exon 2 or within highly conserved gene positions predicted worse survival. Conversely, among comorbidities, cancer history was independently associated with longer survival.

Interpretation Within the context of an overall slower disease, *SOD1*-ALS exhibits some degree of heterogeneity linked to the considerable genetic diversity arising from the multitude of potential mutations sites and specific clinical prognostic factors, including cancer history. Revealing the factors that modulate the phenotypic heterogeneity of SOD1-ALS could prove advantageous in improving the efficacy of upcoming therapeutic approaches.

Keywords SOD1 · ALS · Amyotrophic lateral sclerosis · Genotype–phenotype · Population-based register · Prognostic factors

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Introduction

SOD1 is the first gene identified in Amyotrophic Lateral Sclerosis (ALS), accounting for 15–20% of familial ALS (fALS) and 1–2% of sporadic ALS (sALS) cases [1]. Approximately 200 mutations have been described (https://alsod.ac.uk/, accessed on December 2022), mostly inherited with an autosomal dominant pattern [2]. ALS associated with *SOD1* mutations (*SOD1*-ALS) has been described as characterized by a predominantly lower motor neuron involvement, with limbs onset [3], and a nearly intact cognitive profile [4]. Among *SOD1* mutations, certain variants are associated with distinct disease trajectories [5], ranging from aggressive forms linked to the A5V variant to milder ones due to D91A [4].

According to the multistep hypothesis, starting from genetics, the interaction with other individual and external factors also affects survival in ALS [6]. For example, a noticeable milder disease progression has been observed in female *SOD1*-ALS patients [7]. This epidemiological observation is reinforced by a meta-analysis of preclinical data showing a female hormone-related protective effect in the SOD1 G93A mouse model [8], although this effect is not consistent across all *SOD1* mutations [9].

On the other hand, an older age of onset has been found to be independently linked to a more rapid disease progression [9], although other possible factors influencing the disease trajectory remain unclear. In fact, besides a recent large study on ALS Online Database investigating some basic factors possibly associated with age at symptom onset and survival, to date the majority of genotype–phenotype data pertaining to SOD1-ALS is derived from small monocentric cohorts or individual case reports [5].

Moreover, despite extensive literature data on experimental paradigms, SOD1 role in ALS pathogenesis is not definitively clarified, although most studies favor a toxic gain-of-function mechanism involving protein aggregation and a prion-like propagation of misfolded molecules [10]. Accordingly, an antisense oligonucleotide targeting SOD1 has been developed [11, 12] and is currently being administered through an early access program in many countries (NCT04972487) and has recently gained FDA approval.

In the present study, we aimed to analyzing clinical features and the genotype–phenotype correlates in a cohort of Italian *SOD1*-ALS patients accrued by the Emilia Romagna (ERRALS) [13] and Piemonte and Valle D'Aosta (PARALS) [14] Registers. Furthermore, by gathering medical history from these two regional Italian ALS registries, our study is meant to unravel which factors, among clinical and genetic features and comorbidities, may influence disease progression and survival in *SOD1*-ALS.

Patients and methods

Study population

Our study is designed as a retrospective observational study, involving ALS adult patients (age ≥ 18 years) residing in Emilia Romagna and Piedmont and Valle D'Aosta regions, respectively enrolled by ERRALS and PARALS registers [13, 14] at the time of diagnosis by caring physicians of the ALS centers of the two regions.

Clinical measures

Demographic and clinical variables that were examined for this study included sex, age at onset, diagnostic latency, family history for ALS and/or fronto-temporal dementia (FTD), site of onset (bulbar, upper limb or lower limb, respiratory), phenotype (classic, bulbar, upper motor neuron predominant, flail arm, flail leg, and respiratory ALS) [15, 16], and atypical manifestations including extrapyramidal symptoms. Anthropometric measures as weight, height, and Body Mass Index (BMI) at diagnosis, were registered together with "weight loss at diagnosis" defined as the difference in kilograms between the body weight before symptom onset and body weight at diagnosis. ALS Functional Rating Scale revised (ALSFRS-R) total score at diagnosis was collected, calculating progression rate [17] as previously described [18]. Respiratory function as assessed by periodic Forced Vital Capacity (FVC) determination, and time to noninvasive ventilation (NIV), to invasive ventilation (IV), and to percutaneous endoscopic gastrostomy (PEG) were analyzed too.

Cognitive and behavioral impairment in the FTD disease spectrum were evaluated according to Strong criteria [19]. Comorbidities were categorized as hypertension and cardiovascular diseases, dyslipidemia, chronic obstructive pulmonary disease (COPD) and other respiratory diseases, diabetes mellitus, neuro-psychiatric disorders, autoimmune diseases, thyroid diseases, hematological disorders, oncological history, urologic or gastrointestinal disorders, metabolic diseases (including dyslipidemia, hyperhomocysteinemia, hyperuricemia and gout, and obesity) [20]. Riluzole and other treatments were recorded too.

Genetic analyses and SOD1 variants' analysis

Genetic analysis included at least presence/absence of *SOD1, FUS, TARDBP* mutations and *C9ORF72* expansion, as described previously [21]. In a subgroup of patients, further genes were also explored based on clinicians' decisions (usually guided by the presence of family history of ALS

and/or FTD, young age at onset, or comorbid dementia). We classified *SOD1* mutations according to the exon localization. Then, we analyzed amino acid sequence conservation by applying ConSurf (https://consurf.tau.ac.il) [22]. PredictSNP (https://loschmidt.chemi.muni.cz/predictsnp) was used in order to interpret the functional impact of mutations on the SOD1 protein [23].

Statistics

We assessed differences in characteristics across ALS patients by using *T* test, ANOVA, Chi-square test as appropriate. We reported missing data as a separate category in the dataset and each variable has been described with frequencies of "unknown" values. Cox regression analysis has been used to estimate the hazard ratio (HR) and corresponding 95% confidence interval (95% CI) associating independent variables and ALS tracheostomy-free survival. Adjusted Cox proportional hazard model for time to event outcome was used to assess *SOD1* mutation effects on survival on the entire ALS population (with *SOD1* mutations and without ALS-related gene mutations). Univariate and multivariate Cox survival analysis were performed to examine the independent effects of several factors on survival of the SOD1 ALS cohort. Data analysis was performed using STATA statistical package 15 (StataCorp. 2017. College Station, TX: StataCorp LLC).

Results

SOD1-ALS patients' clinical features

Out of 2204 genotyped patients from the two Italian registers (ERRALS and PARALS), 55 ALS patients (2.5%) carried *SOD1* mutations. Patients without known mutations in ALS-related genes (wm-ALS) were 1936, whereas 213 patients carried other gene mutations as previously reported [18]. Among this latter group the vast majority was represented by *C9ORF72* expansion (150 patients, 70.42% of patients carrying other gene mutations).

The subgroup of *SOD1* carriers consisted of 25 males and 30 females, resulting in a male-to-female ratio of 0.83. Patients with *SOD1* mutations were younger than other patients (mean age at onset in the *SOD1*-ALS group was 59.91 ± 14.29 years, whereas in the wm-ALS it was 65.04 ± 11.55 years; $p = 0.001$) and reported a family history of ALS or FTD more frequently (45% of patients with *SOD1* mutation versus 12.85% of wm-ALS, $p < 0.001$). There were no differences among the two patient groups in terms of gender, BMI or weight loss at diagnosis, diagnostic delay, presence of FTD or parkinsonism are concerned. Patients with *SOD1*-ALS had more frequently a lower limb onset (37 cases, 67.27%) than wm-ALS (686 cases, 35.47%; $p < 0.001$), whereas the opposite was observed for bulbar onset (5 cases, 9.09% among *SOD1*-ALS and 610 cases, 31.54% among wm-ALS, $p < 0.001$). As far as phenotypes are concerned, the bulbar phenotype was more frequent among wm-ALS (9.09% vs 28.08%, $p < 0.001$), while the flail leg phenotype was more frequent among *SOD1*-ALS (38.18% versus 13.81%, $p < 0.001$). There were no differences in other phenotype features between the two groups.

The prevalence of comorbidities in the analyzed cohort was similar between *SOD1*-ALS and wm-ALS patients, relating to hypertension, diabetes, respiratory, cardiac, gastrointestinal, autoimmune, thyroid, hematological, metabolic disorders. The disease progression rate at diagnosis measured as ALSFRS-R monthly decline was 1.24 ± 1.94 points/month in *SOD1*-ALS patients and 1.47 ± 2.14 points/month in the wm-ALS ($p = 0.403$). Patients with *SOD1* mutations underwent PEG placement less frequently (16.27% vs 40.60%, $p = 0.001$) and later (62.17 months vs 28.88 months, $p < 0.001$), compared to wm-ALS patients. However, there was no significant difference in the utilization of NIV (47.27% vs 46.92%, $p = 0.533$) or IV (12.73% vs 16.45%, $p = 0.580$) between the two groups. The mean tracheostomy free-survival from onset was 69.98 months in *SOD1*-ALS patients and 48.29 months in wm-ALS patients ($p < 0.001$). Out of 55 patients with *SOD1* mutations, 34 (61.81%) died during the study, compared to 1461 out of 1936 wm-ALS (75.50%, $p = 0.020$). Lastly, *SOD1*-ALS patients showed a higher survival probability with respect to wm-ALS patients (HR 0.79, 95% CI 0.67–0.94, $p = 0.006$) (Fig. 1). This difference persisted even after adjusting for possible confounding factors such as region (Piedmont versus Emilia-Romagna: HR 0.79, 95% CI 0.67–0.94, $p = 0.007$), sex (HR 0.79, 95% CI 0.67–0.93, $p = 0.006$), and age at onset (HR 0.75, 95% CI 0.59–0.96, $p = 0.021$). When adjusting for site of onset the difference was attenuated (HR 0.85, 95% CI 0.72–1.00, $p = 0.055$).

SOD1-ALS patients' clinical features according to sex at birth

We observed a more aggressive form of the disease in male patients, since they presented a statistically significant shorter time from onset to IV (95 ± 11.3 months for women, 17.8 ± 8.84 months for men, $p < 0.001$). However, survival time (82.23 ± 62.93 months for women, 55.28 ± 52.5 months for men, $p = 0.094$) and progression rate at diagnosis (2.03 ± 3.05 points/month for men; 1.07 ± 1.05 points/month for women, $p = 0.117$) did not show significant differences between the sexes. Table 1 shows the overall demographic and clinical characteristics of *SOD1*-ALS patients, stratified by sex (Table 1).

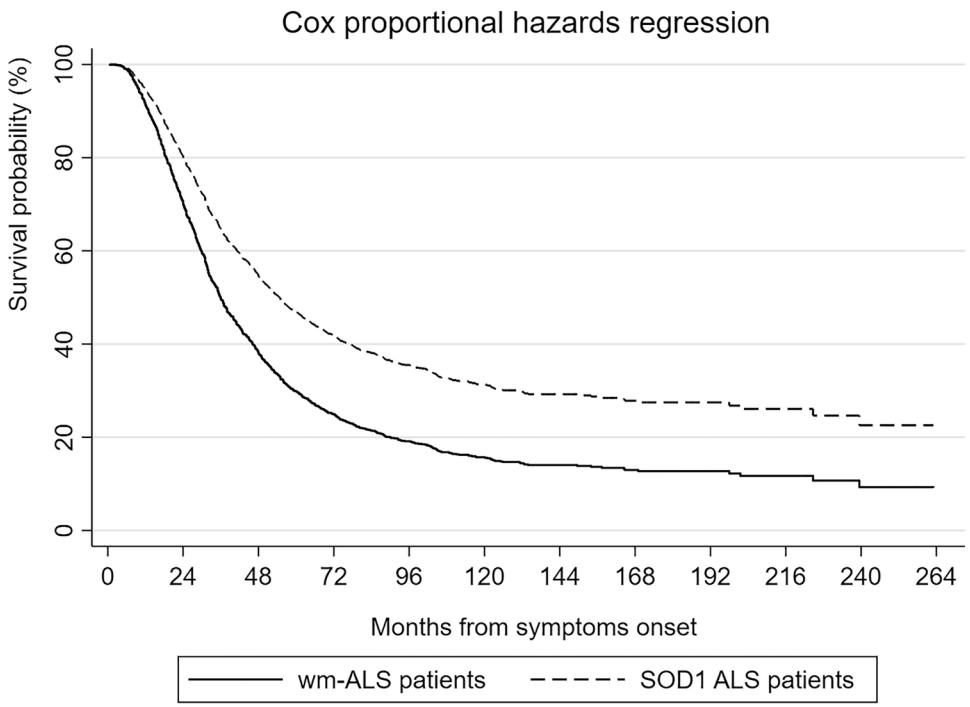

Fig. 1 Cox regression analysis of time to tracheostomy-free survival from symptom onset comparing *SOD1*-ALS and wm-ALS

SOD1-ALS patients' features by *SOD1* mutation

Figure 2 illustrates the localization of SOD1 mutations on the 3D protein structure, while Table 2 represents the demographic and clinical features of *SOD1*-ALS patients by *SOD1* mutation. Some mutations were more prevalent in our cohort, including N66S, D91A, G94D, L145F. While there was a balanced distribution of sex among different mutations, the site of onset and phenotype exhibited heterogeneous representation within each specific mutation group. The site of onset was not only confined to the lower limbs, but also involved the upper limbs and bulbar region. The phenotypic spectrum ranges from the more common flail leg phenotype to the rarer upper motor neuron predominant (UMN-p) or bulbar phenotypes, regardless of the specific type of mutation. A more aggressive disease course, characterized by a mean survival shorter than 1 year, was found in association with L39V (5 months), G42S (6.5 months), G73S (12 months), D91N (9 months), even in presence of a younger age of onset, namely for the L39V (46 years), and G73S (48 years) mutations. None of the patients exhibited symptoms of extra-motor neurological involvement, including cognitive impairment, tremor, or extrapyramidal symptoms (Table 2).

SOD1-ALS patients' survival

The factors influencing survival in *SOD1*-ALS patients, as determined through univariate Cox regression analysis, are presented in Table 3. Besides clinical factors, among the possible prognostic factors related to mutations, the presence of mutations with probable deleterious functional effects (as predicted by Predict SNP software [23]) tended to be associated with shorter survival (HR 1.80, 95% CI 0.99–3.28, $p=0.053$). A statistically significant association with worse survival was finally observed for mutations localized in highly conserved gene positions, as defined by ConSurf software [22] (HR 3.77, 95% CI 1.74–8.23, $p=0.001$), and for mutations located in exon 2 (HR 4.03, 95% CI 1.12–14.40, $p=0.032$) (Table 3).

The multivariate analysis of survival showed that independent prognostic factors related to worse tracheostomy-free-survival were advanced age at onset (years) (HR 1.06, 95% CI 1.02–1.10, $p=0.001$), higher progression rate at diagnosis (> 3 points/month) (HR 1.38, 95% CI 1.15–1.67, $p=0.001$), mutations located in exon 2 (HR 11.56, 95% CI 3.95–33.82, $p<0.001$), and mutations located in highly conserved gene positions, as defined by ConSurf software [22] (HR 4.28, 95% CI 1.80–10.19, $p=0.001$). On the contrary, a history of neoplasms (HR 0.15, 95% CI 0.03–0.69, $p=0.015$) was associated with a longer survival (Fig. 3).

SOD1-ALS patients' features according to the presence or absence of neoplastic diseases

Eight patients had a previous oncological history, with two cases (breast and ovarian cancer) occurring within the same A96T mutation. All the patients with a history of cancer, except one with a benign submandibular adenoma, carried a mutation located in the exon IV and V of

Table 1 Demographic and clinical features of *SOD1*-ALS patients according to sex

Clinical features	Women ($n=30$) *N* (%), mean [SD]	Men ($n=25$) *N* (%), mean [SD]	Total ($n=55$) *N* (%), mean [SD]	*p* value
Mean age at onset, years	61.35 [13.10]	58.20 [15.69]	59.91 [14.29]	0.421
Mean diagnostic delay, months	14.50 [13.54]	12.24 [10.24]	13.47 [12.09]	0.495
Weight loss at diagnosis, kg	3.05 [3.94]	1.71 [2.98]	2.47 [3.58]	0.201
BMI diagnosis, kg/m^2	24.13 [3.67]	26.12 [4.71]	24.96 [4.21]	0.099
Site of onset[a]				0.622
Bulbar	2 (6.67)	3 (12.00)	5 (9.09)	0.412
Spinal, UL	7 (23.33)	5 (20.00)	12 (21.82)	0.514
Spinal, LL	21 (70.00)	16 (64.00)	37 (67.27)	0.426
Phenotype[a]				0.698
Bulbar	2 (6.67)	3 (12.00)	5 (9.09)	0.412
Classic	13 (43.33)	9 (36.00)	22 (40.00)	0.392
Flail arm	0 (0.00)	0 (0.00)	0 (0.00)	
Flail leg	11 (36.66)	10 (40.00)	21 (38.18)	0.509
UMN-p	4 (13.33)	2 (8.00)	6 (10.91)	0.427
Respiratory	0 (0.00)	0 (0.00)	0 (0.00)	
Family history of ALS/FTD	4 (13.33)	5 (20.00)	9 (16.36)	0.380
ALSFRS-R score at diagnosis	39.70 [5.84]	35.95 [12.94]	38.16 [9.50]	0.168
Progression rate at diagnosis[b]	1.07 [1.05]	2.03 [3.05]	1.47 [2.14]	0.117
FVC at diagnosis[c]	109.17 [27.23]	69.70 [39.08]	91.23 [37.50]	0.080
Riluzole treatment	14 (46.67)	9 (36.00)	23 (41.82)	0.345
PEG	3 (10.00)	4 (16.00)	7 (12.73)	0.415
NIV	11 (36.67)	15 (60.00)	26 (47.27)	0.073
IV	2 (6.67)	5 (20.00)	7 (12.73)	0.142
Death	17 (56.67)	17 (68.00)	34 (61.82)	0.389
Time from ALS onset to PEG, months	70.33 [47.81]	54.00 [48.75]	62.17 [44.11]	0.700
Time from onset to NIV, months	54.82 [61.11]	34.27 [28.20]	42.96 [45.24]	0.261
Time from onset to IV, months	95.00 [11.31]	17.80 [8.84]	39.86 [38.63]	**< 0.001**
Time from onset to death or tracheostomy or last observation, months	82.23 [62.93]	55.28 [52.52]	69.98 [59.47]	0.094

BMI body mass index, *Spinal UL* spinal upper limb, *Spinal LL* spinal lower limb, *UMN-p* upper motor neuron predominant, *FTD* frontotemporal dementia, *FVC* forced vital capacity, *PEG* percutaneous endoscopic gastrostomy, *NIV* noninvasive ventilation, *IV* invasive ventilation, *SD* standard deviation

[a]Site of onset and phenotype were available for 54 patients

[b]Progression rate at diagnosis is calculated as monthly decline of ALSFRS-R score assuming a total score of 48 at onset. It was available on 51 patients

[c]FVC at diagnosis was available for 11 patients

SOD1, sparing other mutational regions. Among *SOD1*-ALS patient group, those who reported previous neoplastic diseases did not differ significantly from those with a negative history of neoplasms, except for a longer diagnostic delay (22 ± 20.23 months vs 12.02 ± 9.73 months, $p=0.030$). NIV was more frequently used employed in patients without a history of neoplasm (53.19%) compared to those with a history of cancer (12.5%) ($p=0.023$). The patients' characteristics based on the presence or absence of neoplasia are reported in Table 4.

Discussion

In our cohort, 55 patients with ALS carried a *SOD1* mutation, accounting for 2.5% of the entire genotyped ALS population, a finding consistent with previous reports in European datasets [9, 25]. As expected, patients with *SOD1*-ALS were younger than other ALS patients and exhibited a slower disease progression with longer survival [26, 27]. This group more frequently presented with

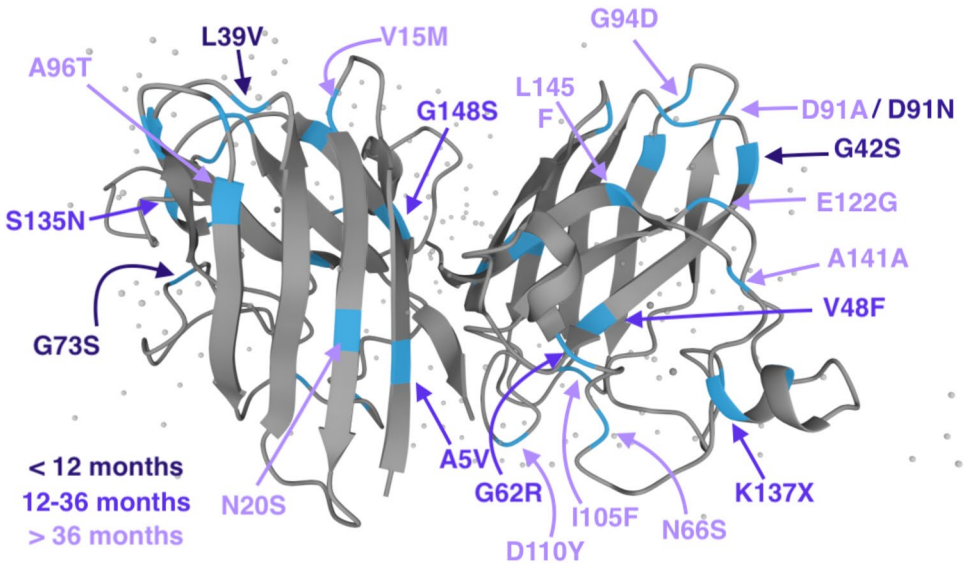

Fig. 2 Genetic variants associated with disease duration plotted onto a wild-type SOD1 protein 3D model. Codon numbers refer to genomic location

lower limb onset and flail leg phenotype. Accordingly, they underwent PEG less frequently and at a later stage compared to patients without mutations in ALS-related genes [28].

Among the possible covariates influencing the association between *SOD1* mutations and longer survival, the onset site emerged as a possible confounding factor, as it was more frequently represented in the *SOD1*-ALS cohort, likely mediating the effect of the SOD1 mutation itself.

However, recent evidence has blurred the clear-cut distinction from the more common ALS presentation by demonstrating how each variant may have a significant impact on the clinical phenotype [4, 5]. For example, the emblematic A5V mutation might be associated with rapidly progressive signs of lower motor neuron involvement in the limbs, trunk, or bulbar-innervated muscles, as well as early respiratory involvement, leading to a limited survival time of 1–2 years [29, 30].

According to a recent study, only a few variants of SOD1 significantly influence survival, with one-third of them appearing to be protective, slowing down overall survival instead of shortening it [5]. In our cohort, the survival of SOD1-ALS patients exhibited wide variability, with an average duration of 70 months, likely attributed to the diverse effects of SOD1 mutations. Previous studies have also demonstrated this heterogeneity in SOD1-ALS survival, as some cohorts reported remarkably long survival periods (e.g., 97.1 months in a Chinese SOD1-ALS cohort) [7], while others indicated comparatively shorter disease durations (e.g., 27.7 and 55.2 months) [5, 30].

Regarding this subject, the primary distinction with Asian cohorts lies in the higher occurrence of the H47R mutation [7, 31], which is not documented in our records, along with the rarity of D91A and A5T mutations [7, 31]. In our population, the most frequently represented mutations within the spectrum of SOD1 variants were N66S, D91A, G94D, and L145F, in alignment with recent findings [5].

Remarkably, in our cohort, we observed a gender difference, with a predominance of female SOD1 cases, whereas other datasets have reported a slight male predominance [5, 7, 30, 32–34]. Additionally, SOD1-ALS patients in our cohort were, on average, 10 years older than those in other studies (60 years versus less than 50 years) [5, 7, 30]. As previously reported, the bulbar phenotype was relatively infrequent, accounting for only 9% of SOD1 cases [5, 7].

Given the small sample size limiting the number of individuals with specific mutations, we analyzed the *SOD1* mutations based on exon localization, the protein amino acid sequence conservation (by ConSurf [22]), and the mutation functional impact (using PredictSNP [23]). Exon 2 localization emerged as an independent predictive factor for worse survival, probably due to two crucial sequences for SOD1 function located here: the dimer interface and the zinc loop. Furthermore, when the mutational point falls within a highly conserved genetic sequence scattered along the gene, regardless of exonic spot, it was associated to a more aggressive form. This observation reinforces Berdynski's findings regarding the assessment of SOD1 variant severity [31]: SOD1 modifications in the highly conserved positions were significantly associated with reduced survival times. These observations suggest a modulation of pathogenetic impact acted by the protein localization, the nature of the amino acid substitution, and the consequent effect on SOD1 dimer stability and the zinc loop. Overall, our data confirm that the selected variants act differently on ALS severity in terms of survival, prompting to further experimental and in silico studies to clarify the intricate relationship between the

Table 2 Demographic and clinical features of *SOD1*-ALS patients according to mutation

Mutation	Exon	No of patient	Sex M/F	Mean age at onset (SD) Years	Mean survival (SD)[a] Months	Site of onset	Phenotype	Cognitive no	Tremor no	Parkinson no	Cancer no/total (localization)
A5V	I	2	2 F	65.88 (23.86)	19.50 (7.78)	2LL	2FL	0	0	0	0/2
V15M	I	1	1 F	78.85 (0.00)	37.00 (0.00)	1LL	1FL	0	0	0	0/1
N20S	I	2	2 F	67.33 (14.50)	66.00 (66.47)	2LL	1FL1UMN	0	0	0	0/2
L39V	II	1	1 F	46.75 (0.00)	5.00 (0.00)	1LL	1FL	0	0	0	0/1
G42S	II	2	2 M	59.34 (19.21)	6.50 (4.95)	1B1UL	1B1C	0	0	0	0/2
V48F	II	2	2 M	26.05 (14.32)	29.00 (22.63)	2UL	2C	0	0	0	0/2
G62R	III	1	1 M	59.08 (0.00)	26.00 (0.00)	1LL	1UMN	0	0	0	0/1
N66S	III	6	3 M 3 F	60.65 (13.26)	97.17 (88.89)	5LL1UL	3C3FL	0	0	0[a]	1/6 (submandibular adenoma)
G73S	III	1	1 M	48.00 (0.00)	12.00 (0.00)	1LL	1FL	0	0	0	0/1
D91A	IV	5	2 M 3 F	66.90 (18.99)	78.00 (46.81)	1B3LL1UL	1B2C 1FL1UMN	0	0	0	1/5 (1 bladder)
D91N	IV	1	1 F	70.75 (0.00)	9.00 (0.00)	1UL	1C	0	0	0	0/1
G94D	IV	6	3 M 3 F	57.83 (14.70)	44.00 (27.85)	5LL1UL	3C3FL	0	0	0	0/6
A96T	IV	2	2 F	55.08 (13.79)	121.00 (94.75)	1LL1UL	2C	0	0	0	2/2 (1 breast 1 ovarian)
I105F	IV	1	1 M	59.83 (0.00)	206.00 (0.00)	1LL	1FL	0	0	0	1/1 (1 kidney)
D110Y	IV	2	2 F	57.09 (0.59)	154.50 (24.75)	2LL	1FL1UMN	0	0	0	0/2
E122G	V	1	1 M	74.50 (0.00)	112.00 (0.00)	1LL	1FL	0	0	0	1/1 (1 lung)
E134del	V	1	1 M	53.17 (0.00)	104.00 (0.00)	1LL	1FL	0	0	0	0/1
S135N	V	2	1 M 1 F	77.17 (3.06)	35.50 (16.26)	2LL	1C1FL	0	0	0	0/2
K137X	V	1	1 M	44.67 (0.00)	14.00 (0.00)	1LL	1C	0	0	0	0/1
A141A	V	1	1 F	51.25 (0.00)	115.00 (0.00)	1B	1B	0	0	0	1/1 (1 uterus)
L145F	V	8	3 M 5 F	55.82 (7.05)	112.88 (40.46)	5LL3UL	4C2FL2UMN	0	0	0	1/8 (skin)
G148S	V	2	1 M 1 F	70.07 (1.24)	29.00 (28.28)	1B11LL	1B1FL	0	0	0	0/2
UK		4	2 M 2 F	65.21 (13.09)	36.75 (18.95)	1B1LL1UL[b]	1B2C[b]	0	0	0[b]	0/4

UK unknown, *M* male, *F* female, *DS* standard deviation, *B* bulbar, *LL* lower limb, *UL* upper limb, *C* classic, *FL* flail leg, *UMN* predominant upper motor neuron

[a]Time to death, tracheostomy or last observation

[b]Unknown

Table 3 Univariate Cox regression analysis of survival in *SOD1*-ALS

Variable	HR (95% CI)	*p* value
Exons and mutation	0.74 (0.58–0.94)	**0.015**
Exon 1 mutations	1	
Exon 2 mutations	4.03 (1.12–14.40)	**0.032**
Exon 3 mutations	0.56 (0.16–1.96)	0.363
Exon 4 mutations	0.40 (0.13–1.22)	0.109
Exon 5 mutations	0.44 (0.15–1.31)	0.140
Variant effect predictor[a]		
Neutral	1	
Deleterious	1.80 (0.99–3.28)	**0.053**
Nucleic acid evolutionary conservation[b]		
Mutation in variable positions	1	
Mutation in highly conserved positions	3.77 (1.74–8.33)	**0.001**
Gender, male	1.68 (0.87–3.25)	0.122
Site of onset	0.72 (0.44–1.19)	0.203
Bulbar	1	
Upper limbs	0.37 (0.12–1.18)	0.094
Lower limbs	0.32 (0.12–0.85)	**0.022**
Phenotype	1.01 (0.83–1.23)	0.916
Classic	1	
Bulbar	3.25 (1.13–9.31)	**0.028**
Flail leg	1.36 (0.64–2.89)	0.417
UMN-p	0.48 (0.11–2.14)	0.334
Weight loss at diagnosis, kg	1.01 (0.90–1.13)	0.853
BMI at diagnosis, kg/m^2	0.96 (0.88–1.05)	0.400
ALSFRS-R score at diagnosis	0.95 (0.92–0.98)	**< 0.001**
Progression rate at diagnosis, points/month	1.29 (1.10–1.50)	**0.001**
Age, years	1.03 (1.00–1.06)	0.062
Diagnostic delay, months	0.94 (0.91–0.98)	**0.006**
Riluzole	0.82 (0.18–3.70)	0.799
Family history of ALS/FTD	0.58 (0.17–2.00)	0.394
Hypertension	1.67 (0.86–3.22)	0.126
COPD	1.40 (0.19–10.36)	0.739
Other respiratory diseases	1.65 (0.38–7.09)	0.501
Heart diseases	1.97 (0.73–5.27)	0.178
Autoimmune disorders	4.92 (0.63–38.44)	0.129
Diabetes	1.19 (0.36–3.89)	0.777
Thyroid diseases	0.71 (0.17–2.95)	0.634
Neuro-psychiatric disorders	0.69 (0.24–1.99)	0.449
Haematological disorders	NA[c]	NA[c]
Neoplastic disease	0.20 (0.05–0.84)	**0.027**
Urologic diseases	< 0.01 (0)[d]	1.000[d]
Gastrointestinal diseases	0.88 (0.31–2.08)	0.654
Metabolic disorders	1.60 (0.56–4.57)	0.378

UMN-p upper motor neuron predominant, *FTD* fronto-temporal dementia, *BMI* body mass index, *ALSFRS-R* ALS Functional Rating Scale-Revised, *COPD* chronic obstructive pulmonary disease, *HR* hazard ratio, *CI* confidence interval

P-values with significance set at <0.05 were highlighted in bold character

Table 3 (continued)

[a]Classification based on PredictSNP software, which classifies the effects of SOD1 mutations as neutral for N20S, D91A, D91N, A96T, D110Y, E122G, and as deleterious for A5V, V15M, L39V, G42S, V48F, G62R, N66S, G73S, G94D, I105F, S135N, L145F, G148S

[b]Classification based on ConSurf software, which classifies the SOD1 mutations based on the evolutionary conservation of the position of the nucleic acid involved by mutation, in a range from 1 to 9, where 5 is average conservation, 1–4 is variable, and 6–9 is highly conserved. Here we considered mutations in highly conserved positions (6 to 9) including A5 (8), L39 (8), G42 (7), V48 (6), G62 (9), N66 (9), G73 (8), G94 (9), A96 (7), S135 (8), K137 (7), A141 (8), G148 (7) versus mutations in variable positions (1 to 5) including V15 (5), N20 (2), D91 (5), I105 (5), D110 (2), E122 (5), E134 (5), L145 (5)

[c]There were not *SOD1*-ALS patients reporting hemotological disorders

[d]Only 1 subject reported urologic disease

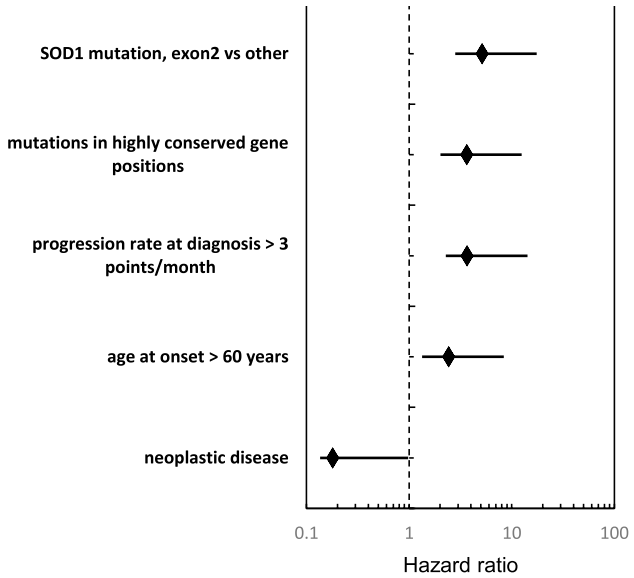

Fig. 3 Forest plots of factors associated with tracheostomy-free survival. The center of the Forest plot represents the hazard ratio of the Cox proportional hazards model, the error bars are two-sided 95% confidence intervals (Cox regression multivariate analysis)

SOD1 molecular modifications and their functional impact on protein structure.

Looking beyond genetics, other clinical variables were associated to survival in our *SOD1*-ALS population. Although in our cohort a more aggressive disease course in men has been initially observed, similarly to what was reported by a Chinese group [7] and reflecting preclinical studies on transgenic (tgG93A-SOD1) mice or rats [35–38], multivariate analysis of survival did not confirm an independent prognostic role for sex in *SOD1*-ALS. The reasons behind these findings could be attributed to the fact that mutations with a poorer prognosis (located in exon 2 and highly conservative domains) were more frequently detected

Table 4 Demographic and clinical features of *SOD1*-ALS patients according to presence or absence of neoplastic disease

Clinical features	Presence of neoplastic disease *N* (%), mean [SD]	Absence of neoplastic disease *N* (%), mean [SD]	Total *N* (%), mean [SD]	*p* value
Sex, male	4 (50.00)	21 (44.68)	25 (45.45)	0.780
Mean age at onset, years	59.97 [10.80]	59.21 [14.90]	59.91 [14.29]	0.991
Mean diagnostic delay, months	22.00 [20.23]	12.02 [9.73]	13.47 [12.09]	**0.030**
Weight loss at diagnosis, kg	1.83 [4.53]	2.56 [3.48]	2.47 [3.58]	0.647
BMI diagnosis, kg/m^2	25.55 [3.21]	24.85 [4.40]	24.96 [4.21]	0.670
Site of onset[a]				0.947
Bulbar	1 (12.50)	4 (8.51)	5 (9.09)	0.559
Spinal, UL	2 (25.00)	10 (21.27)	12 (21.81)	0.563
Spinal, LL	5 (62.5)	32 (68.08)	37 (67.27)	0.524
Phenotype[a]				0.796
Bulbar	1 (12.50)	4 (8.51)	5 (9.09)	0.559
Classic	3 (37.50)	19 (40.42)	22 (40.00)	0.599
Flail leg	4 (50.00)	17 (36.17)	21 (38.18)	0.256
UMN-p	0 (0.00)	6 (12.76)	6 (10.91)	0.370
Family history of ALS/FTD	2 (25.00)	7 (14.89)	9 (16.36)	0.795
ALSFRS-R score at diagnosis	42.12 [3.48]	37.42 [10.09]	38.16 [9.50]	0.201
Progression rate at diagnosis[b]	1.00 [1.84]	1.56 [2.20]	1.47 [2.14]	0.503
FVC at diagnosis[c]	97.17 [25.93]	89.00 [42.38]	91.23 [37.50]	0.766
Riluzole treatment	4 (50.00)	19 (40.42)	23 (41.82)	0.718
PEG	4 (50.00)	3 (6.38)	7 (12.73)	0.223
NIV	1 (12.50)	25 (53.19)	26 (47.27)	**0.023**
IV	0 (0.00)	7 (14.89)	7 (12.72)	0.298
Total	8 (14.54)	47 (85.45)	55 (100)	

Spinal UL spinal upper limb, Spinal LL spinal lower limb, UMN-p upper motor neuron predominant, *FTD* frontotemporal dementia, *FVC* forced vital capacity, *PEG* percutaneous endoscopic gastrostomy, *NIV* non-invasive ventilation, *IV* invasive ventilation, *SD* standard deviation

P-values with significance set at <0.05 were highlighted in bold character

[a]Site of onset and phenotype were available for 54 patients

[b]Progression rate at diagnosis is calculated as monthly decline of ALSFRS-R score assuming a total score of 48 at onset. It was available on 51 patients

[c]FVC at diagnosis was available for 11 patients

in male patients, along with a faster disease progression. It is interesting to note that previous studies also suggest a differential prognosis based on the underlying mutation, considering the limitation arising from different mutational distributions (i.e., the D91A was not present in Chinese case series in ref.7; G38R was absent in our subgroup). In a multicenter Spanish study, female sex was independently associated with faster disease progression in patients carrying the G38R mutation [39]. Conversely, Tang et al. demonstrated worse survival in male patients with *SOD1* mutations when comparing Chinese and German populations [40]. Furthermore, it is noteworthy that disease progression can vary among patients carrying the same *SOD1* mutation but with different haplotypes [41], and co-mutations have been described in SOD1 mutation carriers [42]. These findings suggest that epigenetic factors, interaction with other mutated genes, racial background, and environmental factors may also play a role in the progression of *SOD1*-ALS [39]. In addition to other well-established clinical features associated with SOD1-ALS survival, such as advanced age at onset and progression rate at diagnosis, the history of neoplasm emerged as associated with a slower disease progression, and with a location in the exon IV and V of *SOD1*, sparing other mutational regions. Nevertheless, multivariable analyses confirmed an independent role both of neoplasm history and of location of SOD1 mutation on ALS survival. A selective involvement of exon 5 in patients with history of neoplasms could derive from the crucial role of exon 5 in dismutase activity [43, 44]. Indeed, as a key-player in oxidative stress, previous studies have suggested SOD1 may play a role in tumor development and growth [45]. Beside toxic gain of function characterizing ALS pathogenesis, in the condition of nutrients starvation that characterize cancer cells, SOD1 dephosphorylation and activation enables cancer cells to be

more resistant to oxidative stress and to survive [45]. We could speculate about the presence of variants more eloquent in tumorigenesis pathway and less impactful on ALS progression or a different regulation of mutated SOD1 activity in distinguished tissues/cells. The picture is even more intricate when considering that some SOD1 mutations exhibit incomplete penetrance [46], without a clear association with a specific codon [26], likely arising from alternative splicing [47]. Solving this question was beyond the scope of this study, but we hope that our data will facilitate the design of further experimental and in silico studies aimed at unraveling these complex correlations.

Several limitations should be acknowledged for this study, including its retrospective nature, the small sample size, with some *SOD1* variants very under-represented, and its confinement to Italy, which restricts the applicability to broader populations. Additional studies are required to thoroughly characterize the *SOD1*-ALS variability at both molecular and clinical levels. However, as a cohort study based on disease registers, clinical details were extensively explored within this project, and the results may better reflect the characteristics of the Italian ALS population compared to large studies based on less detailed databases.

Given the lack of prior reports on the impact of concurrent tumor presence on SOD1 ALS survival, it is important to conduct further investigations, utilizing larger prospective cohorts. This approach will help mitigate data instability stemming from our limited sample size. The recent FDA approval of Tofersen, which can be accessed through early access programs (EAPs) in European countries for SOD1-ALS treatment, underscores the significance of systematically offering SOD1 genetic testing. This initiative would not only aid in examining the epidemiological distribution of SOD1-ALS across Western regions but also in gaining a deeper understanding of the diverse phenotypic variations associated with each mutation.

Furthermore, various factors, such as reduced penetrance [48], the occurrence of de novo mutations, or an incomplete family history, should also advocate for a comparable approach in patients with an ostensibly sporadic manifestation of the disease during its early stages. This consideration arises with the hypothesis that early intervention might yield heightened effectiveness, as being investigated in the ATLAS study (NCT04856982) [49]. The comprehension of how each mutation influences the trajectory of SOD1-ALS is of paramount importance for accurately assessing the therapeutic efficacy and potential side effects of gene therapy. Notably, SOD1 antisense oligonucleotides have demonstrated greater effectiveness against rapid disease progression [11]; however, their impact on SOD1-ALS patients with slower progression remains unestablished. Addressing this gap, an open-label trial (NCT03070119) is currently underway.

Lastly, comprehending the extent to which individual and environmental factors contribute to clinical presentation will enhance genetic counseling and the precise interpretation of clinical trial outcomes. The comprehensive analysis of biologically and phenotypically diverse subgroups in clinical studies holds the promise of deeper insights into the survival-related benefits of therapies.

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Availability of data and material Data are available from the authors upon reasonable request.

Declarations

Conflicts of interest The authors have no conflict of interest.

Ethical standard statement This study was approved by the local ethical committee of each participating MND center (Modena Ethical Committe, file number 124/08, on September 2, 2008; Turin Ethical Committe, file number 004462, on June 10, 2010).

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