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ORIGINAL ARTICLE

Characterizing *SOD1* mutations in Spain: The impact of genotype, age and sex in the natural history of the disease

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Abstract

Background and purpose: The aim of this study was to describe the frequency and distribution of *SOD1* mutations in Spain, and to explore factors contributing to their phenotype and prognosis.

Methods: Seventeen centres shared data on amyotrophic lateral sclerosis (ALS) patients carrying pathogenic or likely pathogenic *SOD1* variants. Multivariable models were used to explore prognostic modifiers.

Results: In 144 patients (from 88 families), 29 mutations (26 missense, 2 deletion/insertion and 1 frameshift) were found in all five exons of *SOD1*, including seven novel mutations. A total of 2.6% of ALS patients (including 17.7% familial and 1.3% sporadic) were

See Appendix 1 for all members of ALSGESCO.

estimated to carry *SOD1* mutations. The frequency of this mutation varied considerably among regions, due to founder events. The most frequent mutation was p.Gly38Arg ($n = 58$), followed by p.Glu22Gly ($n = 11$), p.Asn140His ($n = 10$), and the novel p.Leu120Val ($n = 10$). Most mutations were characterized by a protracted course, and some of them by atypical phenotypes. Older age of onset was independently associated with faster disease progression (exp[Estimate] = 1.03 [0.01, 0.05], $p = 0.001$) and poorer survival (hazard ratio 1.05 [1.01, 1.08], $p = 0.007$), regardless of the underlying mutation. Female sex was independently associated with faster disease progression (exp[Estimate] = 2.1 [1.23, 3.65], $p = 0.012$) in patients carrying the p.Gly38Arg mutation, resulting in shorter survival compared with male carriers (236 vs. 301 months).

Conclusions: These data may help to evaluate the efficacy of *SOD1* targeted treatments, and to expand the number of patients that might benefit from these treatments.

KEYWORDS

amyotrophic lateral sclerosis, motor neuron disease, mutation, prevalence, prognosis, *SOD1*

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that typically affects the upper (UMN) and lower motor neurons (LMN). Approximately 10% of ALS patients report a family history of ALS or frontotemporal dementia (FTD) and are considered to have familial ALS (fALS), while 90% have sporadic ALS (sALS) [1]. Mendelian mutations in more than 20 different genes (https://alsod.ac.uk/) have been found to explain approximately 60%–80% of fALS and 10%–20% of sALS [1–3]. The presence of Mendelian mutations in sALS is largely explained by the incomplete penetrance and pleiotropy of many ALS-causing mutations. Moreover, the heritability, at least in some ALS patients, is compatible with an oligogenic origin, where the co-occurrence of two or more mutations is responsible for the emergence and prognosis of the disease [1–4].

An intronic expansion in *C9ORF72* is the most frequent genetic cause of both fALS and sALS in populations of European descent [1], while mutations in *SOD1* are the second cause in those populations and the first cause in non-White patients [5]. More than 200 different mutations, most of them missense and segregating as autosomal dominant mutations, have been described in all five *SOD1* exons in ALS patients (https://alsod.ac.uk/output/gene.php/SOD1). Each mutation is associated with different clinical characteristics regarding penetrance, age of onset, progression rate and survival [6, 7]. However, despite some attempts [8, 9], little is known about the effect of other variables such as age and sex on the prognosis of *SOD1* ALS patients.

Interestingly, the type and frequency of each mutation varies considerably among different regions and countries [7]. Up to now, only one small study has reported the prevalence of *SOD1* mutations in a referral centre in Spain, and no prevailing mutation was identified [10]. With the emergence of gene-based therapies [11, 12], it has become a priority to define the natural history of each mutation (or at least the most prevalent ones). The aim of this study, therefore, was to describe the frequency and distribution of *SOD1* mutations in Spain, and to explore the factors contributing to their phenotype and prognosis.

MATERIAL AND METHODS

Study population and data collection

Through the ALS Genetic Spanish Consortium (ALSGESCO), 29 sites treating ALS patients, and performing genetic studies in them, were contacted by the coordinating centres (Hospital la Fe and Hospital 12 Octubre, H12O). Centres were asked to report their local protocols for genetic study (Appendix S1), the total number of ALS patients in their databases, and the number of fALS cases studied for *SOD1*. Sites were also asked to include data on all previously unpublished ALS patients harbouring pathogenic or likely pathogenic variants in *SOD1* according to the American College of Medical Genetics and Genomics (ACMG) guidelines [13]. To that end, a standardized database was sent to sites. Detailed information on the recorded variables can be found in Appendix S1.

Seventeen centres (mostly referral centres), from 14 different regions, sent charts for this study (Figure 1, Appendix S1). All sites except four have their own patient databases, in which they prospectively collect the clinical and genetic data of ALS patients. Thus, although most patients and data were collected prospectively, data on four patients (and some missing data from other patients) were collected retrospectively.

Data analysis

Demographic, clinical and genetic variables were used to detect duplicates, which were subsequently removed. All variants were

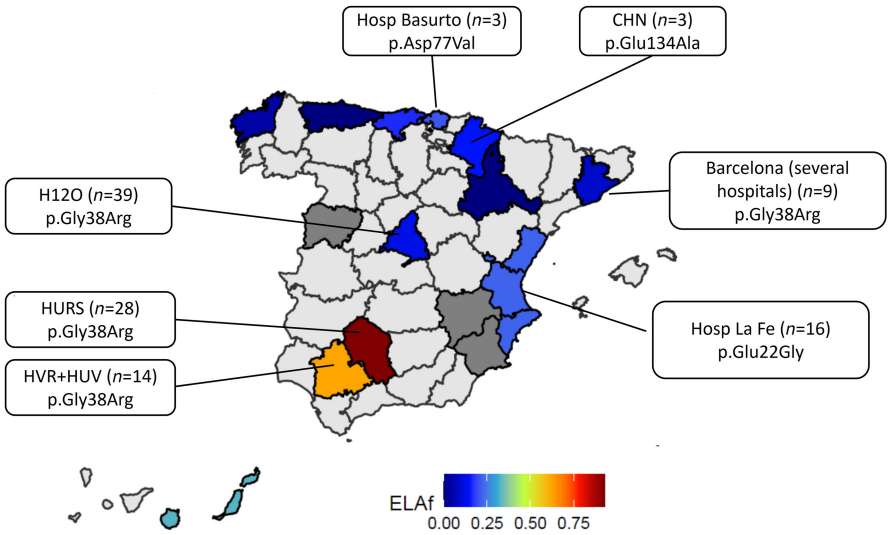

FIGURE 1 Prevalence of *SOD1* mutations among familial amyotrophic lateral sclerosis (fALS) patients in the different regions of Spain. Warmer colours represent higher percentages. Dark grey represents participating regions without their own databases nor prevalence data, while light grey regions did not participate in this study. The number and most frequent mutations found are displayed for the main participating centres. H12O, Hospital 12 de Octubre; HURS, Hospital Universitario Reina Sofía; HVR, Hospital Virgen del Rocío; HUV, Hospital Universitario Virgen de Valme; CHN, Complejo Hospitalario de Navarra.

designated following the new numbering nomenclature, that is, counting the first (ATG) codon of the sequence NP_000445. Several databases (Varsome, MiNE, ALSdb and ALSoD) and publications [3] were reviewed to confirm the ACMG classification and to exclude benign variants and those of unknown significance. According to their penetrance in both our families and the literature [3], variants were classified as having incomplete penetrance (those found mainly in sALS and/or with several obligate carriers) or high penetrance.

Data are summarized using mean, standard deviation, median, and first and third quartiles for the continuous variables, and relative and absolute frequencies for the categorical variables.

The relationships between demographic (sex), clinical (age of onset, phenotype, and progression rate) and genetic (variant and variant penetrance) variables were explored using density plots, boxplots and bar plots. Subsequently, ordinal, linear or gamma regression mixed models were performed to assess those variables influencing the phenotype, age of onset, and progression rate, respectively, adjusting by the random effect of each mutation. Moreover, these models were repeated only for patients carrying the p.Gly38Arg mutation (the most frequently found in our cohort). Kaplan–Meier curves, log-rank tests, and a Cox regression model, including the genetic variant as a random effect, were used to assess the effect of age and sex on tracheostomy-free survival.

All statistical analyses were performed using R software (version 4.1.1).

Ethics approval

Participants had consented for the data collection in their respective centres. Information for all patients was de-identified, and data collection and processing were approved by the Ethics Committee for Biomedical Research of the La Fe Hospital (Valencia) and 12 de Octubre Hospital (Madrid).

RESULTS

Study population and clinical characteristics

Thirteen centres had prospectively collected data on 5252 ALS patients (including 656 fALS patients, 12.5%) in their own registries. *SOD1* mutations were found in 116 fALS patients, representing 17.7% of all fALS. However, the frequency varied considerably between regions, with an important cluster in Southern Spain (Figure 1). Moreover, 23 sALS patients harboured a *SOD1* mutation. In H12O, the only centre that systematically studied *SOD1* in sALS, *SOD1* mutations were found in 14 out of 1048 patients (1.3% of sALS). Two population-based studies found that 7.5% of ALS cases in Spain were fALS and 92.5% were sALS [14, 15]. Based on these figures, 2.53% of all ALS patients (1.33% fALS +1.2% sALS) in Spain would carry *SOD1* mutations.

Five additional patients (one with sALS and four with fALS) were sent by the other four centres without prospective registries. Thus, this study includes 144 *SOD1* patients (120 fALS from 64 families and 24 sALS), 51.4% of whom were female. The mean age of motor symptom onset was 43.6 years, usually in the lower limbs (75.6%) and only rarely in the bulbar region (2.3%). Patients usually showed a slow disease progression rate (DPR; median 0.15 points/month), and a long diagnostic delay (median 17.5 months). At diagnosis, 34.7% of patients showed LMN signs only, and only two patients (1.7%) fulfilled the criteria for FTD. At last follow-up, after a median

of 48.7 months, only 30.3% of patients showed bulbar impairment, 44.4% required ventilatory support and 43.2% had died (median survival 226 months). Further details can be found in Appendix S1.

Genotype–phenotype correlations and geographical distribution

Twenty-nine different pathogenic or likely pathogenic mutations (26 of them missense, 2 deletion/insertion and 1 frameshift) were found in all five exons of *SOD1*, including seven novel mutations in 18 patients of 15 families. Among the novel mutations, two were classified as pathogenic and five as likely pathogenic, according to the ACGM guidelines. The p.Glu41Asp mutation is classified as a variant of unknown significance/likely pathogenic by Varsome and was only found in one sALS patient with LMN-predominant disease and young age of onset. However, this mutation was considered to be likely pathogenic given that: in this patient no other mutations were found after exome sequencing and the *C9ORF72* expansion study; another case with the same mutation (vs. no controls) is reported in the MiNE database; and another missense mutation (p.Glu41Gly) has been reported in the same locus [3]. Altogether, 11 mutations were considered to have high penetrance and 17 incomplete penetrance, based on our own or published data. The main genetic and clinical characteristics of each mutation are summarized in Tables 1 and 2 (full data are provided in Table S1).

The p.Gly38Arg was by far the most frequent mutation in our cohort (40.3%). This mutation was found in 56 patients from 34 kindreds, most of them living in or arising from Southern Spain. p.Gly38Arg caused highly penetrant and early-onset disease (median age 34 years), usually starting in the lower limbs (69%) with a classic ALS (cALS) phenotype (81%) and showing very slow progression and long survival. The second most frequent mutation was p.Glu22Gly (11 patients from six families), mostly found in the Eastern Mediterranean regions of Spain. This variant presents with a similar phenotype to p.Gly38Arg, but with later onset (median age 44 years), strikingly symmetric distance-dependent weakness starting in the lower limbs (100%) and lack of UMN signs (60%). Not surprisingly, before the genetic diagnosis and according to the phenotype and electrophysiological data, some of these patients had been initially diagnosed with distal spinal muscular atrophy (dSMA). The third most frequent variant was p.Asn140His (10 from eight families), also commonly found in the Mediterranean area. This mutation showed a remarkable incomplete penetrance (most cases were sporadic) and phenotype variability, including the site of onset and the prognosis. Specifically, some patients showed a phenotype and family history suggestive of dSMA. Finally, the p.Asp91Ala mutation was found in five patients, in the homozygous state in three of them. Remarkably, one heterozygous patient presented with a Charcot-Marie-Tooth (CMT) phenotype. This patient complained of distal paresthesia, followed by mild weakness in the lower limbs starting at the age of 55 years. The neurological and neurophysiological examination were compatible with a symmetric sensitive-motor axonal polyneuropathy. However, a gene panel targeting all neuropathy and ALS genes only detected a heterozygous p.Asp91Ala mutation.

Among the novel mutations, the most frequent was the p.Val120Leu (n = 10). This mutation was often found in sALS all over the country with a characteristic phenotype: usually starting quite symmetrical in proximal lower limbs and showing a slow DPR with very rare bulbar involvement. Some of these patients did not show UMN signs and, consequently, had been diagnosed with late-onset proximal SMA (pSMA). Another novel mutation, p.Glu134Ala, was found in three patients of the same family, with a variable phenotype but with surprisingly frequent and early cognitive impairment (one patient had FTD and another had mild cognitive impairment at the time of motor symptom onset). All other mutations affected only a small number of patients and were widely distributed throughout the country.

Factors influencing the phenotype and prognosis

Table S2 presents the main characteristics of mutations with high versus incomplete penetrance. Most differences found between them might be largely explained by the high-penetrance p.Gly38Arg mutation, which comprised 40% of the total cohort. To avoid this confounding effect, we represented graphically the most relevant variables in each mutation, considering its penetrance. Figure 2 shows that each mutation is characterized by high heterogeneity regarding age of onset (Figure 2a), degree of UMN impairment (Figure 2b), DPR (Figure 2c) and disease duration (Figure 2d). This appears to be independent of their penetrance, except with regard to DPR, where mutations with high penetrance usually showed faster DPR than mutations with incomplete penetrance. However, an effect of the penetrance in the DPR was not statistically confirmed (Estimate = 0.64 [0.22, 1.88], p = 0.39).

Multivariable models were also performed to analyse the independent effect of demographic factors (age and sex) on the phenotype and prognosis of patients, adjusting for the random effect of each mutation. The same models were repeated only in patients harbouring the p.Gly38Arg mutation to assess the differential effects of demographic factors according to this specific genotype.

Age of onset was not modified by sex, either in the whole cohort or in p.Gly38Arg patients (Estimate = −0.3 [−3.97, 3.42], p = 0.87; and Estimate = 0.89 [−4.26, 6.05], p = 0.73, respectively). Moreover, the LMN phenotype was also independent of sex and age of onset in both the whole cohort (odds ratio [OR] 1.36 [0.57, 3.33], p = 0.48; and OR 0.97 [0.93, 1.01], p = 0.10) and p.Gly38Arg patients (OR 1.3 [0.259, 7.29], p = 0.75; and OR 0.98 [0.9, 1.06], p = 0.56). Conversely, faster DPR was independently associated with older age of onset (exp[Estimate] = 1.03 [0.01, 0.05], p = 0.001), although not with female sex (exp[Estimate] = 1.44 [0.97, 2.12], p = 0.066) in the whole cohort. Moreover, in patients carrying the p.Gly38Arg mutation,

TABLE 1 Main genetic characteristics of the *SOD1* mutations found in our cohort

Variant	N	Number of families	ACMG classification	ALS type	Inheritance pattern	Penetrance
p.Gly38Arg	58	34 (58.62%)	Pathogenic	fALS	AD	High
p.Glu22Gly	11	6 (54.55%)	Likely pathogenic	fALS	AD	High
p.Asn140His	10	7 (70%)	Likely pathogenic	fALS and sALS	AD	Incomplete
p.Val120Leu[a]	10	10 (100%)	Pathogenic	sALS and fALS	AD	Incomplete
p.Asp91Ala	5	3 (60%)	Pathogenic	fALS and sALS	AD (2) and AR (3)	Incomplete/ High
p.Asp77Val	4	3 (75%)	Likely pathogenic	fALS	AD	High
p.Gln23Leu	4	3 (75%)	Likely pathogenic	fALS	AD	High
p.Ile150Thr	4	2 (50%)	Pathogenic	fALS	AD	Incomplete
p.Leu107Val	4	3 (75%)	Likely pathogenic	fALS	AD	High
p.Asn66Ser	3	3 (100%)	Pathogenic	fALS and sALS	AD	Incomplete
p.Glu134Ala[a]	3	1 (33.33%)	Likely pathogenic	fALS	AD	NA
p.Leu85Phe	3	2 (66.67%)	Pathogenic	fALS	AD	High
p.Ser106Leu	3	2 (66.67%)	Likely pathogenic	fALS and sALS	AD	Incomplete
p.Gly94Ser	2	2 (100%)	Pathogenic	fALS	AD	Incomplete
p.Ile113Met	2	1 (50%)	Pathogenic	fALS	AD	High
p.Ile114Thr	2	2 (100%)	Pathogenic	fALS	AD	Incomplete
p.Ile152Thr	2	1 (50%)	Pathogenic	fALS	AD	High
p.Leu118Val	2	1 (50%)	Likely pathogenic	fALS	AD	Incomplete
p.Leu145Phe	2	2 (100%)	Pathogenic	fALS and sALS	AD	Incomplete
p.Arg144Pro[a]	1	1 (100%)	Likely pathogenic	sALS	AD	Incomplete
p.Glu134del	1	1 (100%)	Likely pathogenic	sALS	AD	Incomplete
p.Glu41Asp[a]	1	1 (100%)	Likely pathogenic	sALS	AD	Incomplete
p.His49Arg	1	1 (100%)	Likely pathogenic	sALS	AD	Incomplete
p.Ile113Thr	1	1 (100%)	Pathogenic	fALS	AD	High
p.Leu107_Ser108delinsPro[a]	1	1 (100%)	Likely pathogenic	fALS	AD	High
p.Leu127SerfsTer7[a]	1	1 (100%)	Pathogenic	fALS	AD	High
p.Lys4Glu	1	1 (100%)	Likely pathogenic	sALS	AD	Incomplete
p.Val15Gly	1	1 (100%)	Likely pathogenic	fALS	AD	Incomplete
p.Val6Met[a]	1	1 (100%)	Likely pathogenic	sALS	AD	Incomplete

Note: The order of the mutations in the table reflects its frequency in our population.

Abbreviations: ACMG, American College of Medical Genetics and Genomics; AD, autosomal dominant; AR, autosomal recessive; fALS, familial amyotrophic lateral sclerosis; sALS, sporadic amyotrophic lateral sclerosis.

[a]Novel mutations.

faster DPR was associated with both older age (exp[Estimate] = 1.08 [1.05, 1.12], $p < 0.001$) and female sex (exp[Estimate] = 2.1 [1.23, 3.65], $p = 0.012$).

Finally, the effects of demographic and genetic variables on survival were assessed. The Kaplan–Meier curves and log-rank tests showed longer median survival in patients with p.Gly38Arg than other mutations (292 vs. 122 months, $p < 0.0001$; Figure 3a), while no statistically significant difference was found in male versus female patients (292 vs. 226 months, $p = 0.41$; Figure 3b). Interestingly, when both variables were considered, sex had a differential effect in patients carrying the p.Gly38Arg mutation and in patients carrying other mutations ($p < 0.001$; Figure 3c). Thus, men carrying the p.Gly38Arg mutation showed the longest median survival (301 months), followed by women carrying the p.Gly38Arg (236 months) and women carrying other mutations (142 months), while men carrying other mutations showed the shortest survival (120 months). In the Cox regression models, older age was an independent predictor of poor survival in both patients carrying the p.Gly38Arg mutation (hazard ratio [HR] 1.06 [1.01, 1.12], $p = 0.015$) and the whole cohort (HR 1.05 [1.01, 1.08], $p = 0.007$). Moreover, the differential effect of sex according to the underlying mutation was confirmed in the Cox models; after adjusting for age, female sex still showed a strong (although not statistically significant) deleterious effect on survival in the p.Gly38Arg cohort (HR 3 [0.95, 9.50], $p = 0.062$).

TABLE 2 Genotype–phenotype correlation of the *SOD1* mutations found in our cohort

Variant	*N*	AOO Median (1st, 3rd Q)	Female *n* (%)	Site of onset[a]	Phenotype[a]	DPR Median (1st, 3rd Q)	Bulbar impairment *n* (%)	Respiratory impairment *n* (%)	Cognitive impairment (%)
p.Gly38Arg	56	32.4 (25.4, 39.2)	28 (48.28%)	LL, UL, both	cALS, LMN and UMN	0.07 (0.03, 0.15)	13 (31.71%)	12 (28.57%)	MCI (2%)
p.Glu22Gly	11	46 (41.2, 56)	6 (54.55%)	LL	LMN and cALS	0.11 (0.08, 0.13)	2 (20%)	5 (50%)	Alzheimer (11%)
p.Asn140His	10	48.7 (46, 54.3)	5 (50%)	LL, UL and bulbar	LMN and cALS	0.43 (0.27, 0.67)	3 (37.5%)	5 (62.5%)	MCI (20%)
p.Val120Leu[b]	10	61 (52.6, 67.2)	5 (50%)	LL and UL	cALS and LMN	0.11 (0.1, 0.16)	1 (14.29%)	2 (33.33%)	NO
p.Asp91Ala	5	54.54 (50, 57)	3 (60%)	LL	LMN	0.06 (0.06, 0.06)	NO	NO	MCI (25%)
p.Asp77Val	4	62.5 (58, 69.3)	3 (75%)	LL and bulbar	LMN and cALS	1.16 (0.87, 1.44)	1 (50%)	2 (100%)	NO
p.Gln23Leu	4	55.2 (44.5, 65.5)	3 (75%)	LL	cALS and LMN	1.32 (1.03, 1.69)	NA	NA	NO
p.Ile150Thr	4	42 (40, 44)	2 (50%)	LL and UL	cALS	0.48 (0.48, 0.48)	1 (100%)	1 (100%)	NO
p.Leu107Val	4	42.9 (40.9, 45.5)	2 (50%)	LL	LMN	1.04 (1.04, 1.04)	NO	2 (100%)	NO
p.Asn66Ser	3	43.2 (39.7, 57.8)	2 (66.67%)	LL	LMN and cALS	0.07 (0.05, 0.09)	NO	1 (100%)	NO
p.Glu134Ala[b]	3	60 (58.5, 67.5)	3 (100%)	LL and UL	cALS, LMN and UMN	0.56 (0.32, 0.57)	NO	1 (33.33%)	FTD and MCI (66.6%)
p.Leu85Phe	3	35.4 (32.5, 38)	2 (66.67%)	LL	cALS	1.1 (1.01, 1.19)	1 (100%)	1 (100%)	NO
p.Ser106Leu	3	52 (46.5, 54)	1 (33.33%)	UL and LL	cALS and LMN	0.33 (0.31, 0.36)	1 (33.33%)	1 (33.33%)	NO
p.Gly94Ser	2	57.4 (53.6, 61.2)	0 (0%)	LL	LMN	0.56 (0.44, 0.68)	NO	1 (100%)	NO
p.Ile113Met	2	46.5 (44.2, 48.7)	2 (100%)	LL	cALS	0.76 (0.51, 1.01)	1 (50%)	2 (100%)	NO
p.Ile114Thr	2	52 (49, 55)	1 (50%)	LL and UL	cALS and LMN	0.33 (0.21, 0.45)	NO	1 (100%)	NO
p.Ile152Thr	2	29.3 (28.8, 29.8)	1 (50%)	LL	cALS	NA	NA	NA	NO
p.Leu118Val	2	43.85 (38.3, 49.4)	0 (0%)	LL	LMN	0.37 (0.37, 0.37)	1 (100%)	NA	NO
p.Leu145Phe	2	51.6 (46.8, 56.4)	1 (50%)	LL	cALS	0.62 (0.62, 0.62)	1 (100%)	1 (100%)	NO
p.Arg144Pro[b]	1	58.3	1 (100%)	both	NA	1.32 (1.32, 1.32)	NA	NA	NO
p.Glu134del	1	55	1 (100%)	LL	LMN	NA	NA	NA	NA
p.Glu41Asp[b]	1	31.6	1 (100%)	NA	LMN	NA	NA	NA	NO
p.His49Arg	1	61	1 (100%)	LL	NA	NA	NA	NA	NA
p.Ile113Thr	1	NA	0 (0%)	NA	NA	NA	NA	NA	NA
p.Leu107_Ser108delinsPro[b]	1	NA	0 (0%)	NA	NA	NA	NA	NA	NA
p.Leu127SerfsTer7[b]	1	57.5	0 (0%)	LL	cALS	1.4 (1.4, 1.4)	NA	NA	NO
p.Lys4Glu	1	50	0 (0%)	LL	cALS	NA	NA	NA	FTD
p.Val15Gly	1	30	0 (0%)	LL	LMN	NA	1 (100%)	1 (100%)	NO
p.Val6Met[b]	1	55.5	0 (0%)	LL	LMN	1.44 (1.44, 1.44)	NO	1 (100%)	NO

Note: The order of the mutations in the table reflects its frequency in our population.

Abbreviations: AOO, age of onset; cALS, classic amyotrophic lateral sclerosis; DPR, disease progression rate; FTD, frontotemporal dementia; LL, lower limbs; LMN, lower motor neuron; MCI, mild cognitive impairment; UL, upper limbs; UMN, upper motor neuron.

[a]In these columns the variables are ordered according to its relative frequency in our cohort.

[b]Novel mutations.

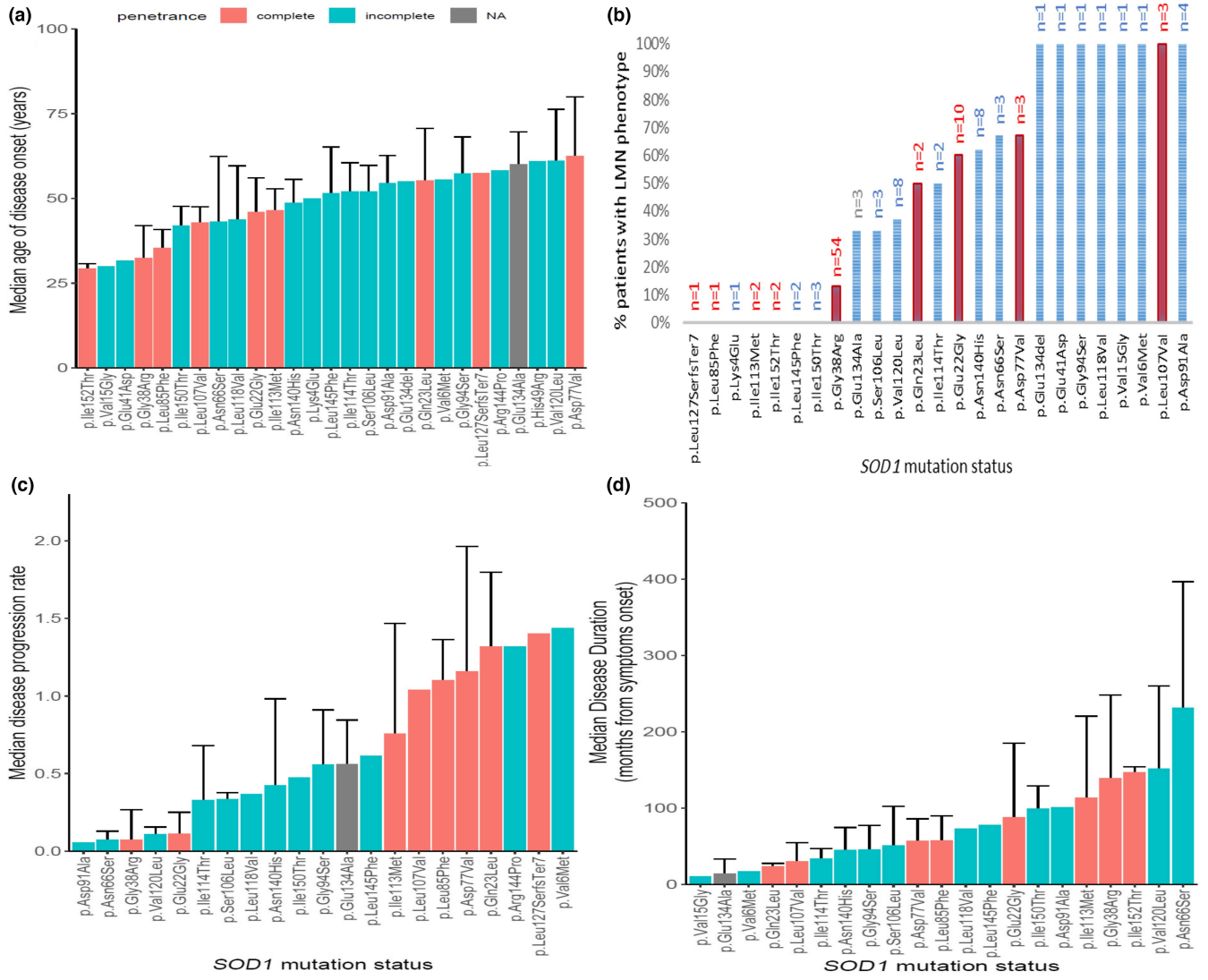

FIGURE 2 Barplots representing the median age of onset (a), proportion of patients with a pure lower motor neuron (LMN) phenotype (b), disease progression rate (c) and disease duration (d), according to the *SOD1* variant. Variants with high penetrance are represented in red and those with incomplete penetrance in blue.

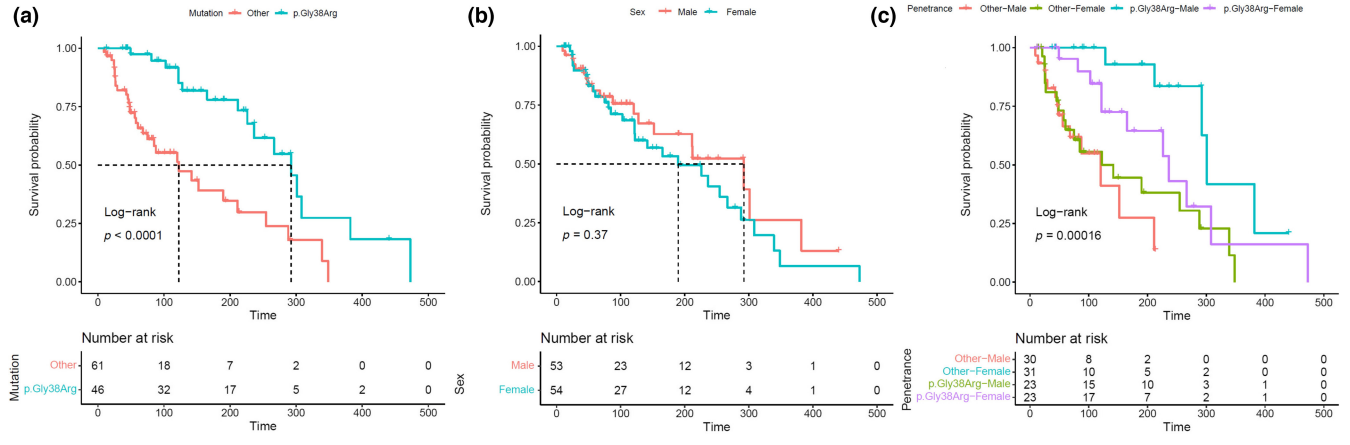

FIGURE 3 Kaplan–Meier curves representing the survival of *SOD1* amyotrophic lateral sclerosis patients according to genotype (a), sex (b), and both variables (c)

DISCUSSION

This multicentre study describes the genetic, epidemiological and clinical characteristics of ALS patients harbouring *SOD1* mutations in Spain.

SOD1 mutations were found in 2.53% of ALS patients in Spain (17.8% of fALS and 1.4% of sALS), representing the second most frequent cause of both fALS and sALS after the *C9ORF72* expansion [16]. These figures are consistent with those found in two small hospital-based studies in Spain [10, 17], in a population-based study in Italy [18] and in a recent meta-analysis in a European population [5]. Considering a population of 47 million in Spain and an estimated prevalence of ALS of 6.89 per 100,000 inhabitants [19], the number of prevalent *SOD1* cases would be 82. Because 63 living patients were identified for this study, we were able to identify 77% of all estimated prevalent SOD1 patients in Spain [10, 18].

Despite showing a similar prevalence, the *SOD1* mutations found in Spain differed considerably from those found in other European studies. Even among the different regions in Spain there were notable differences due to the existence of several founder effects. Moreover, although *SOD1* patients worldwide share some particularities compared with sALS (earlier age of onset, predominant LMN impairment, rare bulbar onset and rare cognitive impairment), there are also substantial disparities in the phenotype according to the underlying mutation. Thus, the most frequent mutations in Spain have a very slow DPR compared to those found in most countries [6, 8, 20, 21]. The description of the phenotype and natural history of each *SOD1* mutation is key in a context where tofersen (an antisense oligonucleotide) has shown promising results [11, 12] and might be approved. This is, firstly, because the response of each mutation to treatment might be different [12] and it will be important to have historical controls to define its efficacy in clinical practice, and, secondly, because the slow DPR found in many mutations (median DPR ≤ 0.11) suggests that outcome measures that are more sensitive than the ALSFRS-R or biomarkers will be needed to prove their efficacy. Indeed, in the recent VALOR study, tofersen achieved a relative stabilization of ALSFRS-R in patients carrying more slowly progressing *SOD1* mutations, but failed to show efficacy in this endpoint, given the slow decline of the placebo group [11].

In this study, we report several mutations with a probable founder event in Spain, including seven novel *SOD1* mutations. Briefly, the highly penetrant p.Gly38Arg mutation was found in 40% of our series and was especially frequent in Southern Spain, showing a classic phenotype with a very protracted course. The p.Glu22Gly also shows high penetrance with a characteristic phenotype: symptoms always start in the lower limbs, quite symmetrically, with an LMN-predominant (pseudo-polyneuritic) phenotype, and progress slowly in a distance-dependent manner, mimicking a dSMA. In fact, only 20% of these patients developed bulbar symptoms through the disease course, while some late-onset patients remained with dSMA phenotypes. It is not surprising therefore, that this mutation has been described in dSMA series [22]. Remarkably, in some individuals, the p.Asn140His mutation also showed a phenotype or a family history suggestive of dSMA, while the p.Asp91Ala mutation was found in heterozygosis in a patient with a CMT phenotype. Finally, the p.Val120Leu, a novel mutation with incomplete penetrance frequently found in our series, usually presented in patients with sALS with proximal weakness in lower limbs followed by proximal upper limbs. The slowly progressive course and the lack of UMN signs in some patients mimicked a pSMA phenotype. In all these cases, other mutations causing those specific phenotypes had been excluded through specific gene panels or clinical exomes. Some studies had reported *SOD1* mutations in patients with atypical phenotypes suggestive of pSMA, dSMA, CMT or spastic paraplegia [20, 23–26], but this study confirms a causal role. Thus, *SOD1* should join the list of genes that can present with a wide spectrum of motor phenotypes (such as *KIF5A*, *CHCHD10*, *VAPB*, *FIG4*) [7, 27, 28], where its analysis should also be considered.

The causes of this pleiotropy are not entirely known. In some genes, a mutational hotspot predisposes to one or another phenotype [27]. In other genes it seems more dependent on other genetic or epigenetic factors because the same mutation can present with different phenotypes in diverse populations [7, 28]. In *SOD1* more than 200 mutations have been described and most of them are associated with a particular phenotype. Our data support previous studies that linked every *SOD1* mutation with a certain phenotype, but the effect of the specific mutations seems to be independent of their exon location or specific penetrance (Table S1, Figure 2). Recent studies suggest that some of this heterogeneity may be explained by the existence of distinct conformations that the SOD1 protein can adopt to produce the equivalent of prion strains [29].

Even recognizing that the huge heterogeneity of *SOD1* patients can in part be explained by the effect of each mutation, there are still considerable differences among patients harbouring the same mutation. Interestingly, the age of onset and disease progression were found to vary in patients carrying the same *SOD1* mutation but different haplotypes [30], and co-mutations have been described in patients harbouring *SOD1* mutations [31], suggesting that polygenic, epigenetic and environmental factors may also have a role in the disease onset and progression of *SOD1* patients. Previous studies have suggested higher penetrance of *SOD1* mutations in men [6, 9]. However, in our cohort we found no evidence of an effect of sex on penetrance, at least in the most frequent mutations. We did not find an effect of sex on either age of onset or phenotype. Notwithstanding, in patients harbouring the p.Gly38Arg mutation, female sex was independently associated with faster DPR and women showed shorter survival. This effect on DPR and survival was not found when the whole cohort was assessed. Interestingly, another work found worse survival in male *SOD1* patients [9], again suggesting a differential effect of sex on the prognosis according to the underlying mutation. Furthermore, older patients showed faster DPR and shorter survival, independent of the underlying mutation, as has also been found in sALS. Thus, our data suggest that the underlying mutation, age of onset and sex should be considered when assessing the efficacy of *SOD1* targeted treatments.

Our study is one of the largest published series, which expands the previous knowledge on *SOD1* by describing new mutations and phenotypes, and providing a comprehensive overview of their natural history and modifying factors. However, this study also has some limitations. Firstly, it was a multicentre retrospective study and, consequently, there is some risk of bias or missing information, including selection bias. Nevertheless, there was a widespread geographical representation and centres were invited to participate regardless of the presence of *SOD1* patients in their databases. Furthermore, epidemiological data were calculated considering previous population-based studies and all efforts were made to collect all possible data and to standardize these according to current criteria. Secondly, most mutations were found in very few patients and 40% of patients carried the p.Gly38Arg mutation. Thus, the results of the models could be overinfluenced by this mutation. To limit this effect, we used mixed models, adjusting for the random effect of every mutation.

In conclusion, *SOD1* mutations are a frequent genetic cause of sALS and fALS in Spain and are characterized by high geographical and clinical heterogeneity and an overall protracted course. The data presented here may help to evaluate the efficacy of *SOD1* targeted treatments in both clinical trials and clinical practice, and to expand the number of patients that might benefit from these treatments.

AUTHOR CONTRIBUTIONS

Juan F. Vázquez-Costa, Alberto García-Redondo and Ricardo Rojas-Garcia are founding members and coordinators of the ALSGESCO, which was supported and funded by CIBERER. Juan F. Vázquez-Costa and Alberto García-Redondo conceived and coordinated the study design, collected the data designed the study, participated in data acquisition and interpretation, and edited the manuscript. Juan F. Vázquez-Costa performed the data analysis and drafted the manuscript. All authors participated in clinical data acquisition, reviewed the paper critically, contributed to scientific and intellectual content and approved the final manuscript. Co-investigators had a minor role in clinical data acquisition.

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CONFLICT OF INTEREST

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DATA AVAILABILITY STATEMENT

Anonymized data not published within this article will be made available by request from any qualified investigator.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX 1

Co-investigators from ALSGESCO

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