A Natural History Comparison of SOD1-mutant Patients with Amyotrophic Lateral Sclerosis between Chinese and German Populations

Lu Tang
Peking University Third Hospital https://orcid.org/0000-0002-3589-6287
Johannes Dorst
University of Ulm: Universitat Ulm
Lu Chen
Peking University Third Hospital
Xiaolu Liu
Peking University Third Hospital
Yan Ma
Peking University Third Hospital
Kornelia Günther
University of Ulm: Universitat Ulm
Sebastian Michels
University of Ulm: Universitat Ulm
Kathrin Müller
University of Ulm: Universitat Ulm
Axel Freischmidt
University of Ulm: Universitat Ulm
Jochen H. Weishaupt
University of Ulm: Universitat Ulm
Dongsheng Fan (dsfan2010@aliyun.com)
Peking University Third Hospital https://orcid.org/0000-0002-3129-9821
Albert C. Ludolph
University of Ulm: Universitat Ulm

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Abstract

Background: The gene coding the Cu/Zn superoxide dismutase (*SOD1*) was the first-identified causative gene of amyotrophic lateral sclerosis (ALS), and the second most common genetic cause for ALS worldwide. The promising therapeutic approaches targeting *SOD1* mutations are on the road. The purpose of the present study was to compare the mutational and clinical features of Chinese and German patients with ALS carrying mutations in *SOD1* gene, which will facilitate the strategy and design of *SOD1*-targeted trials.

Methods: Demographic and clinical characteristics were collected from two longitudinal cohorts in China and Germany. Chinese and German patients carrying *SOD1* mutations were compared with regard to mutational distribution, age of onset, site of onset, body mass index (BMI) at diagnosis, diagnostic delay, progression rate, and survival.

Results: A total of 66 Chinese and 84 German patients with 69 distinct *SOD1* mutations were identified. The most common mutation in both populations was p.His47Arg. It was found in 8 Chinese and 2 German patients and consistently showed a slow progression of disease in both countries. Across all mutations, Chinese patients showed a younger age of onset (43.9 vs 49.9 years, p=0.002), a higher proportion of young-onset cases (62.5% vs 30.7%, p<0.001) and a lower BMI at diagnosis (22.8 vs 26.0, p<0.001) compared to German patients. Although riluzole intake was less frequent in Chinese patients (28.3% vs 81.3%, p<0.001), no difference in survival between populations was observed (p=0.90). Across both cohorts, female patients had a longer diagnostic delay (15.0 vs 11.0 months, p=0.01) and a prolonged survival (248.0 vs 60.0 months, p=0.005) compared to male patients.

Conclusions: Our data demonstrate the distinct mutational and clinical spectrums of *SOD1*-mutant patients in Asian and European populations. Clinical phenotypes seem to be primarily influenced by mutation-specific, albeit not excluding ethnicity-specific factors. Further large-scale transethnical studies are needed to clarify determinants and modifiers of SOD1 phenotypes.

Introduction

Amyotrophic lateral sclerosis (ALS) engenders adult-onset, progressive degeneration of both upper (UMN) and lower motor neurons (LMN), leading to muscle weakness, paralysis, and death usually within 3–5 years. The worldwide all-age prevalence in 2016 was 4.5 per 100,000 people, while the all-age incidence was 0.78 per 100,000 people-years[1]. No effective treatment for ALS is currently available, despite the limited efficacy of riluzole [2] and edaravone [3].

While the majority (roughly 90–95%) of ALS cases are sporadic, a family history can be confirmed in the remaining cases. Increasing numbers of genes responsible for ALS have been identified in recent years; among those, the gene coding the Cu/Zn superoxide dismutase (*SOD1*) was the first ALS-causative gene found in 1993 [4], and accounts for approximately 15–20% of familial and 1–2% of sporadic ALS cases in Germany [5]. *SOD1* mutations are the second most frequent genetic cause for ALS after *C9orf72* in

patients with European ancestry, while it is the most frequently mutated gene in Asian populations [6]. To date, multiple therapeutic approaches have targeted *SOD1*-related ALS. Most importantly, the antisense oligonucleotide tofersen showed promising results in a recent phase I/II trial [7], significantly reducing the SOD1 concentration in the cerebrospinal fluid. Given the clinical heterogeneity among different *SOD1* mutations, it is important to clinically characterize distinct mutations in order to evaluate the efficacy of *SOD1*-targeting therapies. Moreover, the increasing evidence of ethnicity-based epidemiologic and genetic differences motivated us to describe and compare disease-causing mutations and related phenotypes between Asians and Caucasians.

Methods

Participants Inclusion and Variables of interest

Chinese patients were recruited at a national referral motor neuron disease (MND) center at Department of Neurology, Peking University Third Hospital (PUTH), Beijing between 2007 to 2013, which has been described previously [8]. Roughly about one fifth of the patients in this center were recruited from Beijing metropolitan area [9]. German patients were collected from the database of the German network for MND between 1999 and 2019. The German MND network is a consortium consisting of 21 centers specialized in ALS. The ALS center of PUTH and the German ALS network have established a long-lasting cooperation over the last years, which included the synchronization of databases in order to obtain comparable data [10].

Written informed consent was obtained from all patients for participation in clinical and genetic studies, which were approved by the respective local institutional ethics committees. Patients were diagnosed with definite, lab-supported probable, probable, or possible ALS according to Airlie House diagnostic criteria [11] and had a known *SOD1* mutation, a SOD1 variant of uncertain significance, or a likely pathogenic SOD1 variant according to American College of Medical Genetics (ACMG) Standards and Guidelines [12]. Patients were followed up every 3–6 months (Germany: visits in outpatient clinics; China: phone call follow-ups).

Demographic information included sex, date of birth, month/year of disease onset, date of diagnosis, date of last follow-up, month/year of death or invasive ventilation (if applicable, both were defined as endpoint events), and self-reported ethnicity and family history of ALS. Clinical information included site of onset, first clinical symptom, predominant affection of UMN or LMN, body mass index (BMI) at onset, and ALS-Functional Rating Scale Revised (ALSFRS-R) [13] at each visit/follow-up.

Diagnostic delay was defined as the interval between onset (first paresis) and diagnosis. Disease progression rate was defined as loss of ALSFRS-R score per month be[8]tween diagnosis and first visit/follow-up (early progression rate) as well as between first and last visit/follow-up (late progression rate). Survival was defined as the time between onset and endpoint events.

DNA sequencing and analysis

DNA was extracted from blood samples collected from outpatient visits or in hospital, usually at first visit. For Chinese cases, both apparently sporadic patients and the probands of families were consecutively included in genetic analysis. Sequence analysis has been previously partly published[8]. Briefly, Sanger sequencing was performed for all coding exons and flanking 50bps of *SOD1* (NM 000454.5). In Germany, patients with a positive family history of ALS and patients with unusual features (such as young age of onset) were routinely tested. Additionally, sporadic patients without these features who wished genetic testing via the German MND network were tested as well. The patients were screened by Sanger sequencing for all coding exons of *SOD1*(NM 000454.5). The novel variants identified in the present study were evaluated according to the ACMG Standards and Guidelines.

Statistical analysis

Descriptive statistics (frequencies and percentages, mean and 95% confidence intervals [95% CI], or medians and interquartile ranges [IQRs], as appropriate) were used to characterize the study participants. The Chi-square test was used for nominal variables. Independent-sample student's *t*-test or ANOVA test was applied for analyzing normally distributed continuous variables, and non-parametric Mann-Whitney U test or Kruskal-Wallis test for non-normally distributed variables. Kaplan-Meier curves and log-rank test were applied to determine the effect of demographic or clinical parameters on survival. A Cox multivariate proportional hazards model was applied to account for prognostic variables. Statistical significance was set at $p < 0.05$ (two-sided). Because of the explorative nature of this study, the results from the statistical analysis have to be interpreted as hypothesis generating only but not as confirmatory. No adjustment for multiple testing was applied.

Results

The final dataset consisted of 66 (44%) Chinese and 84 (56%) German patients with *SOD1* mutations. The overall male-female ratio was 1.3:1. The mean (95% CI) age of onset was 47.2 (45.2–49.1) years (Chinese: 43.9 [41.6–46.2], German: 49.9 [47.0-52.8], p = 0.002), median (IQR) diagnostic delay was 12.0 (6.0–35.0) months (Chinese: 14.5 [6.0-36.5], German: 11.0 [6.0–32.0], p = 0.59), and median (IQR) survival was 141.0 (21.0-364.0) months (Chinese: cannot be calculated because of more than 60% censored cases at censoring date March 31, 2019, 21 months as the 3nd quartile; German: 198.0 [22.0-364.0]; p = 0.90). The proportion of familial to seemingly sporadic ALS patients was higher in Germany compared to China (84.8% vs 71.2%, p = 0.047).

Mutational Distribution and Characteristics Among exons

A total of 69 distinct SOD1 known mutations, variants of uncertain significance, and likely pathogenic variants were found, among which 89.4% (59/66) in Chinese patients and 98.8% (83/84) in German were reported previously (Supplementary Table 1). Overall, the most frequent mutation was p.Arg116Gly in 26 patients (all German), followed by 11 with p.Asp91Ala (all German) and 10 with p.His47Arg (8 Chinese, 2 German). P.His47Arg was therefore the most frequent mutation present in both populations. Other common mutations seen in both populations included p.His44Arg (3 Chinese and 2 German), p.Leu85Phe

(2 Chinese and 1 German), p.Glu41Gly, p.Asn87Ser, p.Ile114Thr and p.Gly148Asp (1 Chinese and 1 German, respectively). All common mutations featured consistent phenotypes, including an aggressive form of ALS in p.Gly148Asp and slow progression forms in p.Glu41Gly, p.His47Arg and p.Asn87Ser (Table 1).

Table 1
Clinical characteristics of common mutations (≥ 2 patients) in Chinese and German ALS patients

	Number of Patients		Age of Onset (years, mean ± SD)		Survival (months, mean ± SD)	
	Chinese	German	Chinese	German	Chinese	German
Found in both populations						
p.Glu41Gly	1	1	36	41	71*	162 *
p.His44Arg	3	2	40.7 ± 2.9	58 *	38.3 ± 43.3 *	9 *
p.His47Arg	8	2	50.8 ± 10.1	45 ± 14.1	108 ± 55.5 *	210 ± 12.7 *
p.Leu85Phe	2	1	38 ± 0	33	42 ± 2.8	14
p.Asn87Ser	1	1	51	46	82 *	100 *
p.Ile114Thr	1	1	38	64	18 *	63 *
p.Gly148Asp	1	1	43	58	10	7
Found only in Chinese patients						
p.Val15Met	2		54.5 ± 7.8		59.0 ± 14.1 *	
p.Gly17Ala	2		35 ± 8.5		106 ± 24.0 *	
p.Gly42Asp	3		46.7 ± 3.8		67 ± 34.0 *	
p.Gly42Ser	3		43.3 ± 11.5		15.7 ± 7.1 *	
p.Val48Ala	2		51.5 ± 3.5		29 ± 24.0 *	
p.Leu107Phe	3		39.7 ± 5.7		58.5 ± 55.9 *	
p.Cys112Tyr	2		27.5 ± 10.6		88 ± 94.8 *	
p.Gly142Ala	3		48.3 ± 5.7		34.3 ± 8.0 *	
p.Ile150Val	2		42 ± 0		95.5 ± 84.1 *	
Found only in German patients						
p.His49Arg		2		48 ± 7.1		203.5 ± 227.0
p.Gly73Ser		3		45.5 ± 26.2		45 ± 48.1 *
p.Val88Ala		2		61 ± 0		35.5 ± 2.8
p.Asp91Ala		11		48.9 ± 16.0		107.9 ± 62.6 *
p.Glu101Lys		5		37 ± 8.2		188.2 ± 151.6 *

*includes censored data (at least 1 patient still alive or missing data); SD, standard deviation.

	Number of Patients	Age of Onset (years, mean ± SD)	Survival (months, mean ± SD)
p.Ile105Phe	2	42.5 ± 20.5	264.5 ± 20.5 *
p.Ile113Thr	2	65 ± 7.1	15 ± 8.5
p.Arg116Gly	26	52.4 ± 10.5	28.0 ± 21.2 *
p.Leu145Phe	6	51.3 ± 6.4	53.8 ± 30.0 *
p.Val149Gly	2	51.5 ± 3.5	10 ± 2.8
*includes censored data (at least 1 patient still alive or missing data); SD, standard deviation.			

The mutational frequency among the five exons of the *SOD1* gene was different between Chinese and German patients ($p < 0.001$, Fig. 1A) as the most common mutations in Chinese patients were located in exon 2 while those in German patients were mainly located in exon 4. While the average age of onset in patients with mutations in exon 2 was similar between Chinese and Germany, there was a significant difference of average age of onset between Chinese and German patients who carried mutations in exon 4 (37.4 vs 49.9 years, $p < 0.001$, Fig. 1B). Diagnostic delay and survival (Fig. 1C) were not significantly different between the exons ($p = 0.59$ and $p = 0.64$, respectively).

Comparison of Clinical Features by ethnicity

Generally, the Chinese population featured an earlier age of onset of 43.9 years (95%CI 41.6–46.2) than that in the German population (49.9, 47.0-52.8, $p = 0.002$; Table 2) as mentioned above. In addition, juvenile ALS (defined as age of onset < 25 years)[14] as uncommon as it was found in only two Chinese and one German patient. However, the proportion of young-onset ALS, defined as onset between 25 and 45 years[14], was 47.5% (62.5% Chinese and 30.7% German, $p < 0.001$), including 39.5% of males and 52.4% of females ($p = 0.101$). Elderly-onset ALS (> 70 years) was present only in five German cases (4 males and 1 female) and no Chinese patients.

Table 2
Clinical characteristics of *SOD1* mutation populations

	Total (data available)	**China**	**Germany**	**P value****	**Male**	**Female**	**P value****
Nominal variables, n (%)							
Numbers of subjects	150	66	84		80	64	
Sex, male	80 (55.6%) (144)	35 (53.8%)	45 (57.0%)	0.70	-	-	-
Familial ALS	114 (78.6%) (145)	47 (71.2%)	67 (84.8%)	**0.05**	66 (83.5%)	46 (71.9%)	0.09
Juvenile ALS (< 25 years)	3 (2.2%) (139)	2 (3.1%)	1 (1.3%)	-	2 (2.5%)	1 (1.6%)	-
Young-onset ALS (25 ~ 45 years)	63 (47.5%) (139)	40 (62.5%)	23 (30.7%)	**< 0.001**	30 (39.5%)	33 (52.4%)	0.10
Site of onset, spinal	116 (94.3%) (123)	57 (91.9%)	59 (96.7%)	0.25	66 (94.3%)	50 (94.3%)	0.99
Pure LMN	16 (20.0%) (80)	9 (17.3%)	7 (25.0%)	0.41	8 (20.0%)	8 (20.0%)	1.00
riluzole	54(53.5%) (101)	15 (28.3%)	39 (81.3%)	**< 0.001**	32 (60.4%)	22 (45.8%)	0.14
Continuous variables, mean (95%CI)							
Age of onset (years)	47.2 (45.2–49.1) (139)	43.9 (41.6–46.2)	49.9 (47.0-52.8)	**0.002**	48.7 (46.0-51.4)	45.3 (42.5–48.1)	0.08
BMI at diagnosis	24.1 (23.2–24.9) (91)	22.8 (21.9–23.7)	26.0 (24.7–27.4)	**< 0.001**	24.4 (23.3–25.4)	23.8 (22.4–25.2)	0.49
Continuous variables, median (IQR)							

* Follow-up period is the time between diagnosis and last visit/telephone contact (months).

** χ^2 test for nominal variables; *t* test for nominally distributed continuous variables; Mann-Whitney U test for non-nominally distributed continuous variables; log-rank test for overall survival.

Bold p-values are significant.

LMN: lower motor neuron

	Total (data available)	China	Germany	P value**	Male	Female	P value**
Diagnostic delay (months)	12.0 (6.0–35.0) (107)	14.5 (6.0-36.5)	11.0 (6.0–32.0)	0.59	11.0 (5.0-24.8)	15.0 (8.0–47.0)	**0.01**
ALSFRS-R at diagnosis	41.0 (35.0–45.0) (116)	42.0 (35.5–46.0)	40.0 (31.0–44.0)	**0.04**	41.0 (35.0–45.0)	40.0 (34.0–44.0)	0.35
Early progression rate (onset to first visit)	0.42 (0.14–0.90) (116)	0.33 (0.15–0.90)	0.46 (0.13–0.93)	0.79	0.49 (0.20–0.82)	0.31 (0.13-1.00)	0.45
Late progression rate (first to last visit)	0.26 (0.09–0.79) (69)	0.28 (0.08–0.80)	0.17 (0.11–0.77)	0.89	0.30 (0.11–0.90)	0.20 (0.09–0.53)	0.32
Survival (months)	141.0 (21.0-364.0) (140)	NA	198.0 (22.0-364.0)	0.90	60.0 (17.0-250.0)	248.0 (44.0-419.0)	**0.005**
Follow-up period*	24.0 (7.3–40.8) (88)	30.0 (10.0–42.0)	15.0(6.0–40.0)	0.06	13.0 (7.0–31.0)	30.0(9.0–45.0)	0.12
* Follow-up period is the time between diagnosis and last visit/telephone contact (months).							
** χ^2 test for nominal variables; *t* test for nominally distributed continuous variables; Mann-Whitney U test for non-nominally distributed continuous variables; log-rank test for overall survival.							
Bold p-values are significant.							
LMN: lower motor neuron							

No differences regarding diagnostic delay, early or late progression rate between both populations were observed as shown in Table 2; while the ALSFRS-R at diagnosis of Chinese patients (median 42.0, IQR 35.5–46.0) was higher than that of German patients (40.0, 31.0–44.0, p = 0.04; Table 2). Despite lower riluzole prescription rates in the Chinese population (28.3%) compared to the German population (81.3%, p < 0.001), no survival differences determined by log-rank test were observed between both populations (p = 0.90, Table 2).

The majority of cases (94.3%) had spinal onset, while there was no proportional difference between Chinese and German patients (91.9% vs 96.7%, p = 0.25) or between male and female patients (94.3% vs 94.3%, p = 0.99). 20% of patients featured a pure LMN phenotype (Chinese: 17.3%, German: 25.0%, p =

0.41; Table 2). Some of these patients progressed slowly (p.His47Arg), while others showed an aggressive pattern (p.Ala5Val and p.His44Arg) (Table 3). With regard to BMI, we found a significant difference between Chinese (mean 22.8, 95% CI 21.9–23.7) and German patients (26.0, 24.7–27.4, $p < 0.001$), but no difference between males and females (males: 24.4, 23.3–25.4; females: 23.8, 22.4–25.2; $p = 0.49$) (Table 2)

Table 3
Patients carrying *SOD1* mutations with pure LMN presentation

Patient No.	Group	Mutaion	Sex	Hereditary	Age at onset (years)	Survival (months)
1	China	p.Ala5Val	Male	fALS	51	7
26	China	p.His44Arg	Male	fALS	39	19 *
28	Germany	p.His44Arg	Male	fALS	58	9
33	China	p.His47Arg	Female	fALS	38	112 *
34	China	p.His47Arg	Male	fALS	54	95 *
37	China	p.His47Arg	Female	fALS	40	203 *
48	Germany	p.Gly73Ser	Female	fALS	64	79 *
58	Germany	p.Asn87Lys	Male	fALS	45	118 *
65	Germany	p.Asp91Ala	Female	sALS	41	21 *
74	China	p.Gly94Arg	Female	fALS	34	55
86	China	p.Leu107Phe	Male	fALS	33	98 *
92	China	p.Cys112Tyr	Male	sALS	20	155 *
93	China	p.Cys112Tyr	Female	fALS	35	21 *
97	Germany	p.Ile114Thr	Female	fALS	64	63 *
138	Germany	p.Leu145Phe	Male	fALS	57	75 *
144	Germany	p.Val149Ala	Female	fALS	68	45

* Still alive or lost at the last following-up.

Male patients featured a significantly shorter diagnostic delay (median 11.0 months, IQR 5.0-24.8) and survival (median 60.0 months, IQR 17.0-250.0) compared to females (diagnostic delay: 15.0, 8.0–47.0, $p = 0.01$; survival: 248.0, 44.0-419.0, $p = 0.005$). In German patients, these differences were not significant (diagnostic delay: male 11.5, 4.8–29.8; female 11.0, 8.0-47.5, $p = 0.50$; survival: male 72.0, 19.0-250.0; female 248.0, 41.0-419.0; $p = 0.15$); while these differences by sex in median diagnostic delay (male 9.5, 4.8–24.3; female 24.0, 9.5–47.5; $p = 0.009$) and median survival (male 42.0; $p = 0.009$, Fig. 2) were

significant in Chinese patients. Due to censored cases in 49% males and 73% females, the median survival and IQR of Chinese females and IQR of Chinese males could not be calculated.

Prognostic factors of survival

To evaluate the effect of age of onset on survival, we formed four subgroups based on quartiles (Chinese: 38.3, 43.0, and 50.0 years, German: 41.0, 50.0, and 58.0 years), which did not reveal any significant differences ($p = 0.33$, Fig. 3A). Subgroups of quartiles for BMI did not reveal any effect of body weight on survival (Chinese: 20.9, 22.6, and 24.9, German: 23.1, 25.9 and 28.7; $p = 0.97$, Fig. 3B). Likewise, we did not detect any significant effects of age of onset and BMI on survival in each population (Fig. 4). Patients with bulbar onset had a worse prognosis compared with spinal onset ($p = 0.001$, Fig. 3C). Survival of patients with pure LMN signs was not significantly different from typical ALS phenotypes who featured clinical signs of both UMN and LMN degeneration ($p = 0.16$, Fig. 3D).

Further, in the univariate regression analysis (Table 4), several factors indicated in present analyses and previously-known prognostic factors were found to be predictive for poor prognosis, including male sex (hazard risk [HR] 2.10, $p = 0.006$), bulbar onset (HR 3.74, $p = 0.003$), higher early progression rate (HR 1.15, $p = 0.009$), and higher late progression rate (HR 2.77, $p < 0.001$), while a better prognosis was associated with a prolonged diagnostic delay (HR 0.93, $p < 0.001$). Because of the significant relationship between diagnostic delay, early progression rate, and late progression rate (data not shown), only sex, age of onset (HR 1.02, $p = 0.054$), site of onset, and late progression rate were included in the multivariate Cox regression analysis (Table 4), which revealed that patients with bulbar onset (HR 10.31, $p = 0.01$) and higher late progression rate (HR 2.42, $p = 0.003$) had a much shorter survival time.

Table 4
Cox regression analysis of *SOD1*-mutant patients

	Univariate		Multivariate	
Variable	**HR (95%CI)**	**p Value**	**HR (95%CI)**	**p Value**
Ethnicity				
Chinese	1.00			
German	1.04 (0.61–1.75)	0.90	-	-
Sex				
Female	1.00			
Male	2.10 (1.23–3.57)	**0.006**	1.77 (0.60–5.21)	0.30
Age of onset (years)	1.02 (1.00-1.04)	0.054	1.01 (0.97–1.06)	0.68
Site of onset				
Spinal	1.00			
Bulbar	3.74 (1.58–8.86)	**0.003**	10.31 (1.70-62.54)	**0.01**
Diagnostic delay (months)	0.93 (0.90–0.96)	**< 0.001**	-	
BMI (kg/m^2)	0.96 (0.87–1.05)	0.34	-	
Early progression rate (point/month)	1.15 (1.04–1.27)	**0.009**	-	
Late progression rate (point/month)	2.77 (1.64–4.67)	**< 0.001**	2.42 (1.36–4.28)	**0.003**
riluzole	1.00 (0.52–1.90)	1.00	-	-

Discussion

This study constitutes the first direct comparison of mutational and clinical characteristics of Asian and Caucasian ALS patients with *SOD1* mutations based on two large hospital-based registry cohorts. Overall, 150 patients carrying 69 distinct mutations, of which 7 mutations were found in both populations, did not show survival difference between the two populations.

Our findings demonstrate a significant lower age of onset in the Chinese *SOD1*-mutant patients compared with their German counterparts (43.9 vs 49.9 years). The Chinese age of onset was therefore also younger compared to *SOD1*-mutant patients from Canada (48.9 years)[15] and the United States (46.9–49.7 years) [16, 17]. A previous bi-ethnic comparison showed that an earlier age of onset in Chinese compared to German patients was also present and even more pronounced in sporadic ALS (51.0 vs 61.0, $p < 0.0001$)[10]. In the present study, both Chinese and German *SOD1* patients featured a much higher

proportion of young-onset cases (62.5% and 30.7%, respectively) compared to 10% in the overall ALS population[14]. Moreover, young-onset ALS was significantly more frequent in Chinese compared to German *SOD1* patients ($p < 0.001$). An earlier age of onset is hypothesized to reflect a higher burden of genetic and environmental risk factors[18]. Our results of a generally young age of onset of *SOD1*-mutant patients are in line with the multistep model of ALS[19], which proposes that the number of steps in ALS was 2 in patients with *SOD1* mutations, 3 in patients carrying *C9orf72* expansions, and 6 in patients without known mutations. The age of onset in the whole ALS population varies between different ethnicities : In Asia (India: 46.2 years, China:52.4 years[20]), Africa (approximately 50 years[21]), and South America (Rio de Janeiro/Brazil: 53.6 years[22], Cuba and Uruguay: 54.4 and 59.5 years[23]) age of onset is younger compared to Europe (Germany: 61.0 years[10]; Ireland: 63.0 years[23]) and North America (US: 61.5 years[24]). A systematic review[25] of ALS cohorts showed considerable differences regarding the mean ages of onset among patients carrying distinct mutations: The youngest ages of onset were found in patients with *VCP* (11.6 years), *hnRNPA1* (33 years), *ALS2* (37.4 years), and *FUS* (41.8 years) mutations, the oldest in Fig. 4 (61.3 years), *TUBA4A* (59.6 years), and *TBK1* (59.5 years). Therefore, our results confirm *SOD1*-specific features (*SOD1*-mutant patients featuring a younger age of onset in both cohorts compared to the overall ALS population) while at the same time also displaying ethnicity-specific features known from sporadic ALS (younger age of onset in Chinese compared to German patients).

A striking 95% of *SOD1* cases in the present study displayed spinal onset which is significantly higher compared to the prevalence of spinal onset in the whole ALS population, which is about 70%-80%[25] and consistent across both countries. In the whole ALS population, a lower proportion (16%) of bulbar onset is present in patients with young onset (< 41 years) compared to older onset (> 70 years, 43%)[26]. Thus, the high share of young onset ALS in our cohort could partly explain the high proportion of spinal onset. Consistent with previous findings of spinal onset constituting a positive prognostic factor in the whole ALS population, we found a significant difference regarding survival between spinal-onset and bulbar-onset patients carrying *SOD1* mutations ($p = 0.001$).

A notable proportion of 20% of cases in our cohort featured pure LMN signs. Several ALS-associated genes have been described to be associated with a proportion of > 20% of patients with pure or predominant LMN signs (*hnRNPA1, SETX, SOD1, and TBK1*)[25]. On the other hand, both juvenile ALS, usually caused by mutations of *FUS, ALS2, SETX* (*ALS4*), and *ALS5* genes, and young-onset ALS have been found to feature predominantly UMN rather than LMN dysfunction[14]. For *SOD1*, specific mutations have been reported to result in severe LMN phenotypes. The mutation A4V (p.Ala5Val according to former nomenclature), accounting for up to 50% of *SOD1*-related fALS cases in the United States, causes a rapidly progressive form of pure LMN presentation with a median survival of 1.2 years[17]. This is also the case in the Chinese patient carrying an A4V mutation in our study. A p.D12Y mutation also caused an LMN-predominant presentation[27]. Interestingly, in *SOD1-mutant* patients, pathological signatures of SOD1 protein inclusion staining have been mainly found in LMN rather than UMN[28].

The finding of significantly lower BMIs of Chinese *SOD1*-mutant patients compared to their German counterparts (mean 22.78 vs 26.00, $p < 0.001$) is consistent with previous findings in sporadic ALS (mean 23.0 vs 24.5, $p < 0.0001$)[10]. BMI is an indicator of nutritional status, and malnutrition is a prognostic factor of survival in ALS patients[29]. In the whole Caucasian ALS population, patients with below-normal BMI have a shorter median survival compared to those with normal BMI[24]. Metabolic changes have increasingly been recognized in ALS, and hypermetabolism has been identified as the potential main reason for loss of body weight[30]. Experimentally, mutant SOD1 protein can cause mitochondrial dysfunction and metabolic imbalance[31].

We have confirmed several previous known prognostic factors in this *SOD1* cohort, i.e., site of onset (spinal vs bulbar) and disease progression rate (especially late progression rate). Of note, some known prognostic factors in the whole ALS population, including BMI and age of onset, were not associated with survival, indicating that the spectrum of disease-modifying factors may be different from sporadic ALS. Another possibility is that there is not a simple linear relationship between BMI, age of onset, and survival in patients carrying *SOD1* mutations. Interestingly, in *SOD1*-G93A murine models of different genetic backgrounds (C57BL/6J and 129S2/Sv), animals carrying the same mutation and expressing the same amount of mutant *SOD1*-G93A protein have been found to feature significantly different disease progression rates and survival[32], highlighting a set of potential key genes and molecular pathways acting as disease modifiers and possibly contributing to the heterogeneity of ALS phenotypes[33]. It is therefore reasonable to assume that both known specific mutations and unknown susceptibility genes might interact with environmental factors to modulate disease phenotypes and prognosis.

Sex has been reported as an independent factor affecting ALS vulnerability and phenotypes. In our cohorts, female patients showed significantly longer survival compared to male patients, especially in the Chinese population. In a Dutch case-control study, a longer exposition to estrogen was significantly correlated with a longer survival in female ALS patients[34]. A negative association between incidence of ALS and a dose-dependent use of hormonal contraception also highlighted a possible protective role of estrogen and progestogen[35]. A very recent study from an Italian register cohort suggested an influence of sex, together with age and gene variants, on ALS phenotypes[36]. Evidence from *SOD1* transgenic mice [37, 38] and rat models [39][39][39][39][39] also demonstrated that female animals displayed extended lifespan and delayed onset compared to their male counterparts.

Limitations

Our study is not without limitations. First, our hospital-based cohorts may imply a selection bias compared to population-based studies, i.e., patients of hospital-based registries are known to be younger, predominantly male, and feature a lower share of bulbar onsets[10]. Second, cognitive status was not included in this study, despite that few cases carrying *SOD1* mutations have been reported to feature cognitive dysfunction[25]. Third, potential modifier genes were not analyzed. However, it is not common in *SOD1*-mutant patients to carry another causative gene mutation[40, 41]. On the other hand, we regard

the existence of large synchronized databases and that patients were derived from specialized ALS centers in both countries as main strengths of our study.

Conclusions

Our study demonstrates directly for the first time the mutational and clinical spectrums of *SOD1*-mutant patients in Asian and European populations, identifying distinct mutational distributions in both populations and significant differences regarding clinical features, such as age of onset and BMI at diagnosis. While different genetic mutations featured distinct clinical phenotypes, including disease onset and survival, common mutations in both countries featured rather similar pictures. Furthermore, we found that that some previously known positive prognostic factors in the whole ALS population, such as female sex (especially in China) and spinal onset were present in *SOD1*-mutant patients as well, but others such as young age of onset and high BMI did apparently not influence survival, at least not as prominently. Further large-scale studies in multiple ethnicities are needed to clarify the exact underlying mechanisms and interactions.

Abbreviations

ACMG: American College of Medical Genetics;

ALS: Amyotrophic lateral sclerosis;

ALSFRS-R: ALS Functional Rating Scale–Revised;

ANOVA: Analysis of variance;

BMI: Body mass index;

CI: Confidence interval;

C9orf72: Chromosome 9 open reading frame 72;

FIG4: FIG4 phosphoinositide 5-phosphatase;

FUS: Fused in sarcoma

G93A: Gly93Ala;

hnRNPA1: Heterogeneous Nuclear Ribonucleoprotein A1

HR: hazard risk;

IQR: interquartile ranges;

LMN: lower motor neuron;

MND: motor neuron disease;

PCR: Polymerase chain reaction;

PUTH: Peking University Third Hospital;

SETX: Senataxin;

SOD1: Superoxide dismutase-1;

TBK1: TANK Binding Kinase 1

TDP-43: TAR DNA-binding protein 43;

TUBA4A: Tubulin Alpha 4a

UMN: Upper motor neuron;

VCP: Valosin Containing Protein

Declarations

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Authors' contributions

L.T., J.D., D.F., and A.L. conceived and designed the study. L.T. and J.D. analyzed the data and drafted the manuscript. All authors contributed to the acquisition of data, revising the manuscript and approval of the final version.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Peking University Third Hospital, Beijing, China and the local medical ethics committees of German MND network.

Consent for publication

Not applicable.

Competing interests

None declared.

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Figures

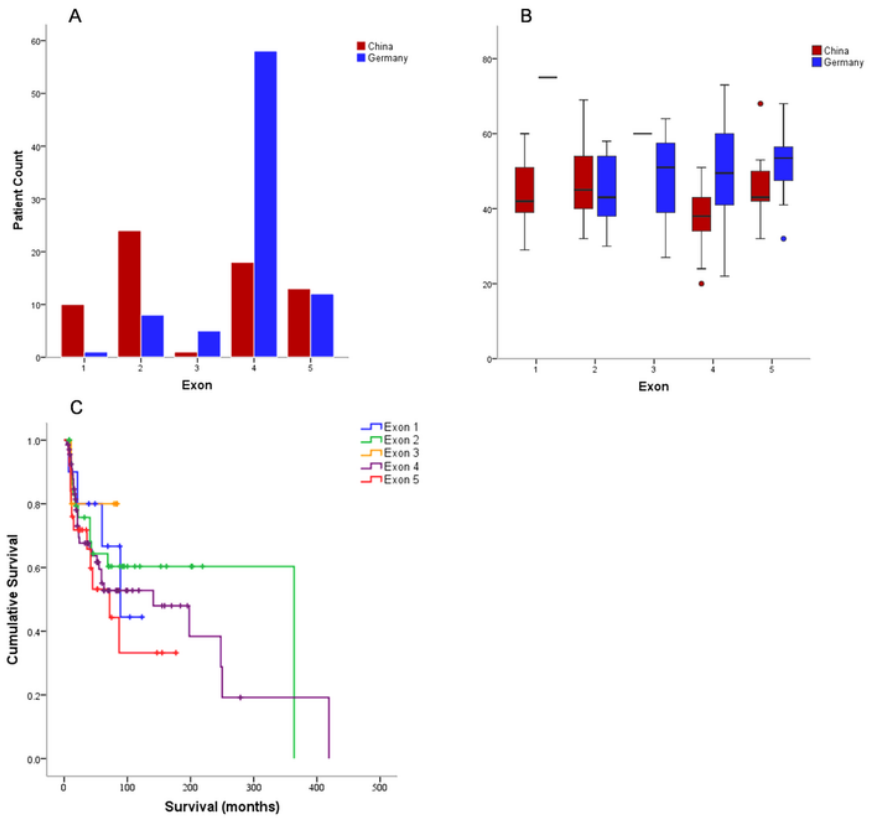

Figure 1

Demographic and clinical features of patients carrying SOD1 mutations by exons. (A) Exonal distribution of patients with SOD1 mutations in China and Germany. The distribution was significantly different ($p<0.001$). (B) Age of onset of Chinese and German patients per exon, revealing a significant difference (*) in exon 4 (China: mean 37.4 years; Germany: 49.9 years, $p<0.001$). (C) Survival curves per exon showed no significant differences ($p=0.64$).

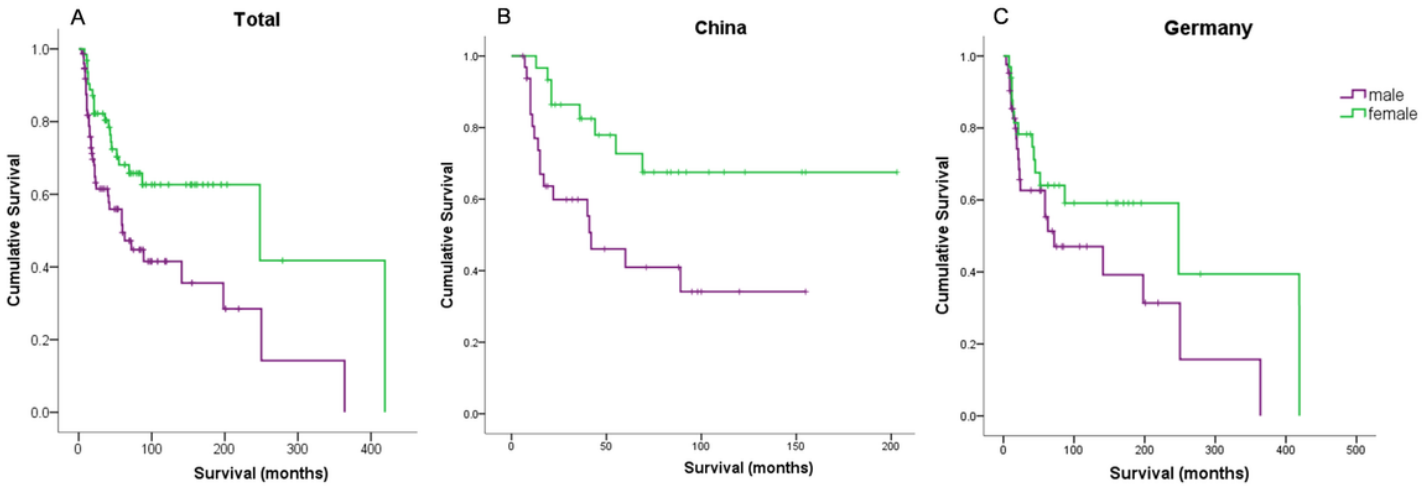

Figure 2

Sex and Survival. Kaplan Meier curves show survival for male (purple) and female (green) patients overall (A: $p=0.005$), in China (B: $p=0.009$), and Germany (C: $p=0.15$).

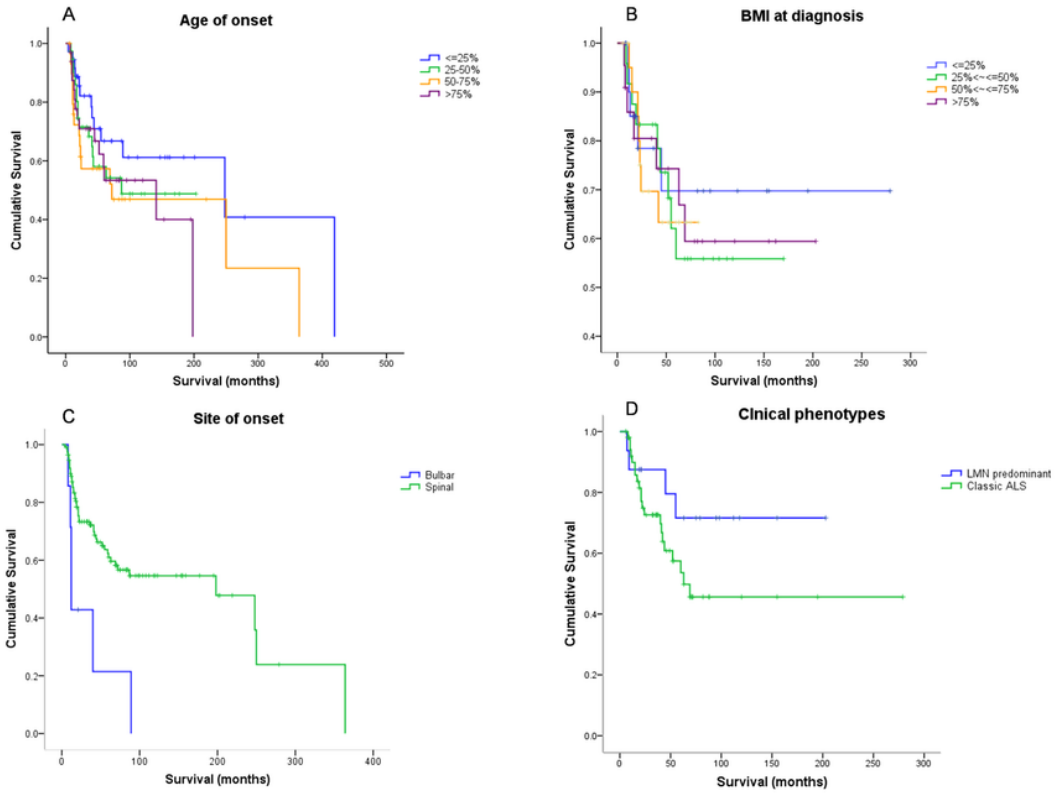

Figure 3

Prognostic factors and survival. Kaplan Meier curves show the effect of age of onset (A, p=0.33), BMI at diagnosis (B, p=0.97), site of onset (C, p=0.001), and clinical phenotype (D, p=0.15) on survival. LMN = lower motor neuron.

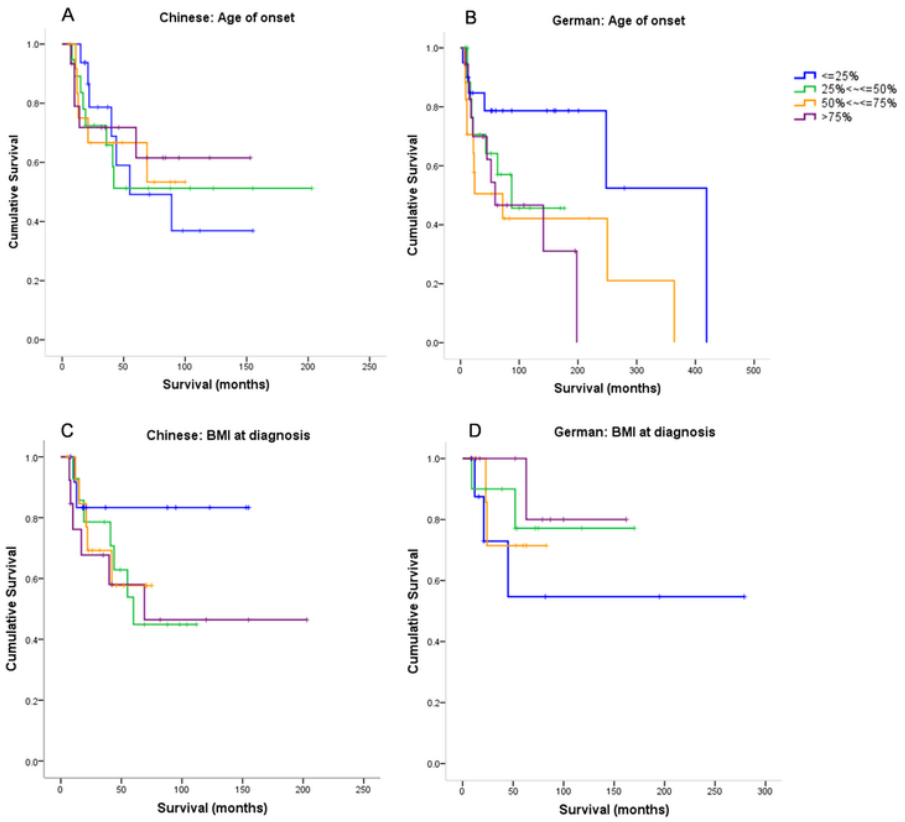

Figure 4

Effect of age of onset (A and B) and BMI (C and D) at diagnosis on survival in China and Germany. Age of onset in Chinese (A: quartiles are 38.3, 43.0 and 50.0 years) and German patients (B: 41.0, 50.0 and 58.0) did not affect survival (Chinese: p=0.80; German: p=0.10). There was also no significant difference of BMI on survival in Chinese (C: quartiles are 20.9, 22.6 and 24.9, p=0.55) and German (D: 23.1, 25.9 and 28.7, p=0.58) patients.

Supplementary Files

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- Supplementarytable1.xlsx