SOD1 mutation spectrum and natural history of ALS patients in a 15-year Cohort in Southeastern China

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Research

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Abstract

Background

Mutations in superoxide dismutase 1 gene (SOD1) are the most frequent high penetrant genetic cause for amyotrophic lateral sclerosis (ALS) in the Chinese population. A detailed natural history of SOD1-mutated ALS patients will provide key information for ongoing genetic clinical trials.

Methods

We screened for SOD1 mutations using whole exome sequencing (WES) in Chinese ALS cases from 2017 to 2021. Functional studies were then performed to confirm the pathogenicity of novel variants. In addition, we enrolled previously reported SOD1 mutations in our centers from 2007 to 2017. The SOD1 mutation spectrum, age at onset (AAO), diagnostic delay, and survival duration were analyzed.

Results

We found two novel SOD1 variants (p.G17H and p.E134*) that exerted both gain-of-function and loss-of-function effects in vitro. Combined with our previous SOD1-mutated patients, thirty-two probands with 21 SOD1 mutations were included with the four most frequently occurring mutations of p.V48A, p.H47R, p.C112Y, and p.G148D. SOD1 mutations account for 58.9% of familial ALS (FALS) cases. The mean (SD) AAO was 46 ± 11.4 years with a significant difference between patients carrying mutations in exon 1 [n = 5, 34.6 (12.4) years] and exon 2 [n = 8, 51.4 (8.2) years] (p = 0.038). The mean of the diagnostic delay of FALS patients is significantly earlier than the sporadic ALS (SALS) patients [9.5 (4.8) years vs 20.3 (9.3) years, p = 0.0026]. In addition, male patients survived longer than female patients (40 months vs 16 months, p = 0.05).

Conclusion

Our results expanded the spectrum of SOD1 mutations, highlighted the mutation distribution, and summarized the natural history of SOD1-mutated patients in Southeastern China. Male patients were found to have better survival, and FALS patients received an earlier diagnosis. Our findings assist in providing a detailed clinical picture, which is important for ongoing genetic clinical trials.

Background

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by the involvement of both upper and lower motor neurons in the spinal cord, brainstem, and motor cortex with or without cognitive dysfunction. ALS insidiously begins with focal weakness and muscle atrophy, but relentlessly spreads to the diaphragm. Eventually the paralysis typically causes death in 3–5 years as a result of respiratory failure. Approximately 90% of ALS cases are sporadic (SALS), and the remaining cases are inherited (familial ALS or FALS) with a Mendelian pattern of inheritance, which implies that a single gene mutation can drive the pathogenesis of ALS.

The advancement of genetic technologies, such as whole exome sequencing (WES), have facilitated the identification of genes associated with ALS. Currently, more than 50 genes have been implicated in ALS. With joint efforts, genetic analysis has added valuable pieces to the ALS puzzle. SOD1 was the first ALS-associated gene dating back to 1993(1), and led to engineering of the first transgenic model of SOD1-G93A mice(2). It ushered in a new era of ALS research and set the stage for future genetic breakthroughs.

SOD1 is a ubiquitously expressed protein, existing as a homodimer of 32 kDa. Each monomer is highly structured, and intramolecular disulfide bridges increase their stability. However, mutations in SOD1 can destabilize the protein and contribute
to the collapse of the homodimeric structure and its subsequent aggregation(3). It is well established that \textit{SOD1} mutations lead to toxic gain-of-function of SOD1 proteins, but the effects of loss-of-function remain controversial(2).

To date, more than 200 mutations in \textit{SOD1} have been reported (http://www.hgmd.cf.ac.uk/). ALS patients carrying \textit{SOD1} mutations present with a highly heterogenous phenotype, and is clinically indistinguishable in SALS and FALS. \textit{SOD1} gene mutations are the leading cause of FALS in the Chinese population. Therefore, it is important to clinically summarize both the mutation spectrum of the \textit{SOD1} gene and the natural history of \textit{SOD1}-mutated ALS patients in Southeastern China.

\section*{Methods}

\subsection*{Participants}

Patients were recruited from the Second Affiliated Hospital of Zhejiang University School of Medicine from May 2017 to April 2021. A total of 114 patients with SALS and 15 with FALS were screened using WES. For further phenotype-genotype analysis, we included previously reported \textit{SOD1}-mutated probands in our centers from December 2007 to April 2017. All patients were diagnosed by at least two senior neurologists as having ALS according to the Revised El Escorial criteria(4). All participants were of Han Chinese descent and came from Southeastern China. The ethics committee of each participating center approved the study, and written consents were obtained from all participants during their first hospital visit. Clinical data of ALS patients, including gender, family history, age at onset (AAO), diagnostic delay, site of onset, side of onset, disease duration, and clinical manifestations were collected upon the first visit to the hospital.

\subsection*{Whole exome sequencing (WES), bioinformatic analysis, and sanger sequencing}

Genomic DNA was extracted from blood using a DNA Extraction Kit (Qiagen, Hilden, Germany). The samples were captured by the Agilent Sure Select Human All Exon V6 products, and sequenced on the Illumina HiSeq X Ten platform (XY Biotechnology Co. Ltd., Hangzhou, China). Further bioinformatic analysis has been performed according to our previously reported protocol(5). Briefly, all variants were annotated by ANNOVAR. SIFT and PolyPhen-2 software were used to predict the functional changes in proteins caused by the variants. Single Nucleotide Polymorphism (dbSNP) Database, the 1000 Genomes Project, and the ExAC database were used to check the frequency in the general population. Variants were finally classified according to the American College of Medical Genetics and Genomics (ACMG) standards and guidelines. Sanger sequencing was performed to validate the potential variants. All primers covering the 5 exons in \textit{SOD1} were seen in our previous report(6).

\subsection*{Plasmid construction}

The coding sequence of human wild-type (WT) \textit{SOD1} gene (RefSeq NM_000454.5) was cloned into the pFlag-CMV-4 vector using a ClonExpress II One Step Cloning Kit (Vazyme). Plasmids with mutant \textit{SOD1} (c.400G>T and c.49_50del insCA) were created by PCR mutagenesis (Toyobo, Osaka, Japan), and were verified by Sanger sequencing. Four plasmids expressing Vector, WT, p.G17H, and p.E134* were constructed.

\subsection*{Cell culture and transfection}

HEK293T cells were maintained in DMEM-supplemented 10% fetal bovine serum stored in a humidified incubator under 5\% CO\textsubscript{2} at 37\textdegree{}C. Transient transfection was performed using Lipofectamine 3000 (Invitrogen, Life Technologies, Grand Island, NY, USA).

\subsection*{Immunofluorescence confocal microscopy}

HEK293T cells were seeded on PDL-treated coverslips (NEST, China) and transiently transfected with various expression plasmids (pFLAG-\textit{SOD1}-WT, pFLAG-\textit{SOD1}-G17H, pFLAG-\textit{SOD1}-E134*). Twenty-four hours later, the cells were rinsed with 1X PBS, fixed with 4\% paraformaldehyde for 8 min, and then permeabilized with 1X PBS supplemented with 0.01\% Triton X100. The cells were blocked with 5\% donkey serum and 1\% bovine serum albumin (BSA; sigma, St.Louis, MO) in PBS for 1 hour at room temperature. The primary antibody was rabbit antibody anti-flag (1:800, Cell Signaling Technology, 14793S).
secondary antibody was Alexa Fluor 488 donkey anti-rabbit. The cells were incubated with NucBlue Live Reagent (Hoechst 33342; Thermo Fisher Scientific, Oregon, USA) and visualized using Olympus FluoView FV3000 confocal microscopy with a 63X objective.

**Western blot**

Forty-eight hours after transfection, HEK293T cells over-expressing pFlag-\(SOD1\)-WT, pFlag-\(SOD1\)-mutants or the empty vector were lysed and harvested. The protein samples were resolved by SDS-PAGE, transferred to PVDF membrane, and blotted with the 5% non-fat milk. The antibodies against flag (1: 1000, CST) and \(\beta\)-tubulin (1: 5000) were used. All immunoblotting images were acquired using a BioRad system.

**Quantitative real-time PCR**

Total RNA was extracted using Trizol reagent (Takara, Kusatsu, Japan), which was then reverse transcribed to cDNA by the PrimeScript RT reagent Kit (Takara, Kusatsu, Japan) in accordance with the manufacturer's instructions. RT-PCR was further performed using a SYBR Premix Ex Taq Kit (Takara, Kusatsu, Japan). The PCR conditions are listed as follows: incubation for 3 min at 50°C followed by incubation for 3 min at 95°C, and finally 40 cycles at a duration of 10 s each while at 95°C and then 30 s at 30°C. The following primers were used: target \(SOD1\) and human \(GAPDH\). Detection and data analysis were conducted using an ABI StepOnePlus sequence detection system (Thermo Fisher Scientific, Oregon, USA), and endogenous \(GAPDH\) was used as an internal control. Expression levels were quantified by threshold cycle values.

**Statistical analysis**

Descriptive statistics were provided for the site of onset, heredity of disease (familial or sporadic ALS), gender, AAO, diagnostic delay and survival time. Besides, continuous data was compared using student's t-test or the Mann-Whitney test, while dichotomous variables, such as gender and site of onset, were analyzed using either the standard chi-square test or Fisher's exact test. Survival duration was determined by Kaplan-Meier analysis, and differences were determined by log-rank testing. A two-tailed \(p < 0.05\) was considered statistically significant. All analyses were performed using GraphPad Prism 8.0 (GraphPad Software, CA, USA).

**Results**

**Whole exome sequencing screen of ALS patients and pathogenicity classification of novel \(SOD1\) mutations**

From 2017 to 2021, a total of 8 different variants in \(SOD1\) were found, including 6 known pathogenic variants (p.G38R, p.V48A, p.N87S, p.C112Y, p.I114T, and p.L145S) and 2 novel variants (p.G17H and p.E134*). Two heterozygous variants, c.49_50delinsCA (p.G17H) and c.400G > T (p.E134*), were detected in two families with a positive family history (Fig. 1A and 1B), which was confirmed with sanger sequencing (Fig. 1C and 1D). Both novel variants were absent in ESP6500 and ExAC database, and were highly conserved from chimpanzee to zebrafish (Fig. 1E and 1F). The nonsense variant, p.E134*, generates the truncated protein caused by premature termination. However, the nonsense variant was not regarded as very strong evidence of pathogenicity because loss of function is not the primary mechanism in \(SOD1\)-related ALS. In addition, p.E134* was predicted to be detrimental by Mutation Taster and CADD. According to ACMG guidelines, the p.G17H variant was classified as likely pathogenic, whereas p.E134* was classified as variant uncertain significance (VUS).

To further elucidate the biological effects of these two variants, SOD1 protein and mRNA levels of mutants were markedly reduced compared to WT (Fig. 1G and 1H), indicating that the reduced protein expression was caused by reduced mRNA synthesis. The *in vitro* results showed that the mRNA levels of mutant \(SOD1\) were degraded more rapidly than WT, suggesting a loss-of-function effect. The aggregation propensity assay showed that the cells overexpressing WT plasmid showed diffuse cytoplasmic SOD1 protein, while the misfolded aggregates were seen in cells transfected with p.G17H and p.E134* mutants (Fig. 1I). The soluble physiological SOD1 protein had a strong tendency to become toxic aggregates due to mutation, implying a gain-of-function effect. Therefore, the pathogenicity of the variant p.E134* was finally confirmed as likely pathogenic.
Mutation frequency of SOD1 in our ALS patients from Southeastern China

In this study, a total of 114 patients with SALS and 15 patients with FALS were screened by WES. Among them, 12 subjects were genetically identified as having SOD1 variants, 10 (10/15, 66.7%) of whom were FALS patients and 2 (2/114, 1.8%) of whom were apparently SALS patients. We previously reported 20 probands with SOD1 mutations in 36 unrelated FALS patients (6–9). When integrating these results into the current study, SOD1 mutations account for 58.9% (32/51) of our FALS cases.

A total of 21 SOD1 mutations, including 1 nonsense and 1 deletion/insertion mutations, were found spanning all five exons, but only one mutation was in exon 3 (Table 1). The most frequent SOD1 mutation was p.V48A (4/32, 12.5%) in 4 FALS probands, followed by p.H47R, p.C112Y, and p.G148D (each were found in 3 probands (3/32, 9.4%)), as well as p.H121Q, p.L145S (each were found in 2 probands (2/32, 6.3%)), and the remaining mutations were each found in 1 proband (Fig. 1J). Among all the mutations, p.L145S, p.P75S, and p.I114T are found in sporadic ALS patients (Fig. 1J).
Table 1
The clinical features of the probands carrying mutations in \textit{SOD1}

| Probands | Variant | Exon | Gender | Family History | AAO (y) | Diagnostic Delay (m) | Site of Onset | Side of Onset | Disease Duration (m) | Predominant Features |
|-----------|---------|------|--------|----------------|---------|----------------------|--------------|--------------|---------------------|---------------------|
| 1         | A5S     | 1    | M      | F             | 29      | 3                    | LL           | L            | 12                  | LMN dominance        |
| 2         | A5V     | 1    | F      | F             | 47      | 8                    | UL           | L            | 15                  | Classical ALS        |
| 3         | G11V    | 1    | F      | F             | 25      | 9                    | LL           | L            | 15                  | Classical ALS        |
| 4         | G17C    | 1    | M      | F             | 49      | 8                    | spinal       | L            | 38                  | LMN dominance        |
| 5         | G17H    | 1    | M      | F             | 23      | .                    | LL           | R            | 36                  | Classical ALS        |
| 6         | F21C    | 1    | M      | F             | .       | .                    | .            | .            | .                   | .                   |
| 7         | G38R    | 2    | M      | F             | 40      | 12                   | spinal       | Both         | >156                | Classical ALS        |
| 8         | H47R    | 2    | F      | F             | 58      | 84                   | UL           | R            | 180                 | LMN dominance        |
| 9         | H47R    | 2    | M      | F             | 53      | 18                   | LL           | Both         | 120                 | LMN dominance        |
| 10        | H47R    | 2    | F      | F             | 55      | 18                   | LL           | Both         | >144                | LMN dominance        |
| 11        | V48A    | 2    | F      | F             | 53      | 20                   | LL           | R            | 17                  | LMN dominance        |
| 12        | V48A    | 2    | F      | F             | 42      | 12                   | UL           | Both         | >30                 | LMN dominance        |
| 13        | V48A    | 2    | F      | F             | 46      | 13                   | LL           | R            | >27                 | Classical ALS        |
| 14        | V48A    | 2    | M      | F             | 64      | 12                   | LL           | Both         | >25                 | LMN dominance        |
| 15        | P75S    | 3    | M      | S             | 59      | 23                   | LL           | Both         | 65                  | LMN dominance        |
| 16        | D84G    | 4    | M      | F             | 32      | .                    | LL           | R            | 70                  | LMN dominance        |
| 17        | N87S    | 4    | M      | F             | 72      | 11                   | UL           | R            | >21                 | Classical ALS        |
| 18        | L107V   | 4    | M      | F             | 41      | 2                    | LL           | R            | 10                  | Classical ALS        |
| 19        | C112Y   | 4    | M      | F             | 47      | 9                    | UL           | R            | 53                  | LMN dominance        |
| 20        | C112Y   | 4    | M      | F             | 50      | 15                   | LL           | R            | 40                  | LMN dominance        |
| 21        | C112Y   | 4    | M      | F             | 47      | 6                    | LL           | R            | 60                  | LMN dominance        |

Abbreviation: AAO = age at onset; UL = upper limb; LL = lower limb; LMN = lower motor neuron
Clinical features and natural history of ALS patients carrying the SOD1 mutation

Combining our previously reported SOD1 mutated patients in our centers, a total of 32 probands were genetically identified with SOD1 mutations (Table 1). 29 (29/32, 90.6%) of the probands were FALS patients, and 3 (3/32, 9.4%) were apparently SALS patients. The gender ratio (M: F) was 1:1. With the exception of 1 case with missing data, only 1 patient among 31 available probands exhibited bulbar onset. The majority of them (18/31, 58%) presented with lower limb onset, but some (10/31, 33.3%) presented with upper limb onset. Sixteen probands presented with a predominantly lower motor neuron phenotype, while another 16 probands were the classical ALS phenotype.

With the exception of the patient with missing data, 31 patients had available AAO data for analysis. The mean (SD) AAO was 46 ± 11.4 years, and the median AAO was 47 years (Fig. 2A). The mean AAO of patients carrying mutations in exon 1 [n = 5, 34.6 (12.4)] was statistically significantly earlier than those with mutations in exon 2 [n = 8, 51.4(8.2)] (p = 0.038) (Fig. 2B). There was no difference in the mean (SD) AAO between male and female patients [47.6 (12.2) vs 45.25 (11.2), p = 0.58] (Fig. 2C). The onset of FALS in patients is earlier than that of SALS, albeit without statistical significance [47.6 (12.2) vs 45.25 (11.2), p = 0.58] (Fig. 2D). The AAOs of ALS patients were highly heterogenous with the 25th and 75th percentiles at 38 and 53 years, respectively. Six mutations (p.A5S, p.G11V, p.G17H, p.D84G, p.G148D, p.I150T) showed relatively younger AAOs, which was lower than the rst quartile (25%, 38 years) and 6 mutations (p.H47R, p.V48A, p.P75S, p.N87S, p.I114T, p.H121Q) presented with relatively older AAOs, which were higher than the third quartile (75%, > 53 years). The youngest and oldest AAOs are p.G17H and p.N87S, which were found in patients in their twenties and seventies, respectively.

Twenty-nine probands patients were accessible for data regarding diagnostic delay. With the exception of one case with an extremely long diagnostic delay of 84 months, the mean (SD) diagnostic delay of the remaining was 10.7 ± 6.2 months.
(Fig. 3A). The diagnostic delay showed no differences in different exons (Fig. 3B). The time to diagnosis of male patients exhibited no difference compared to female patients [10.4 (5.9) months vs 11.0 (6.7) months, p = 0.82] (Fig. 3C). It is worth noting that the mean of the diagnostic delay of FALS patients is less than that of SALS patients, and was found to be statistically significant [9.5 (4.8) months vs 20.3 (9.3) months, p = 0.0026] (Fig. 3D). Diagnostic delay varied greatly across different mutations. Four mutations of 4 probands, namely p.A5S, p.L107V, p.C147R, and p.G148D, showed a diagnostic delay less than the first quartile (25%, ≤6 months). Another 5 mutations of 8 probands, namely p.H47R, p.V48A, p.P75S, p.C112Y, and p.L145S showed a diagnostic delay more than the third quartile (75%, >12 months).

Disease duration data were available for 31 probands, and 9 patients still survive at the censoring date. The median survival time was 40.0 months and the 5-year survival rate was 30.7% for all subjects (Fig. 4A). The survival time showed no difference between all male and female patients (Fig. 4B). Given the extremely long survival time in mutation of p.H47R, we excluded the p.H47R mutation in both male and female patients for statistical analysis. Strikingly, male patients showed significantly longer survival time than female patients (40 months vs 16 months, p = 0.05) (Fig. 4C).

**Discussion**

In this study, which was combined with our previously reported SOD1 mutations, 32 ALS probands were harboring 21 confirmed SOD1 mutations. This is the first study to study SOD1 mutations to date in the Southeastern Chinese ALS population. The extensive analysis of SOD1 mutation distribution and natural history could serve ongoing clinical trials targeted for patients with SOD1 mutations.

Both novel variants (p.G17H and p.E134*) could form toxic aggregates in vitro, indicating that they caused loss of protein intrinsic stability. Interestingly, a loss-of-function effect was also shown in both variants, given the reduced protein expression and mRNA synthesis. Many SOD1 mutations, such as p.G93A, p.G37R, and p.H48Q, would increase protein expression, which is in accordance with the gain-of-function presumption [11]. However, overloading misfolded SOD1 proteins would induce endoplasmic reticulum (ER) stress, subsequently activating the unfold protein response (UPR) to maintain homeostasis. Once the UPR is activated, overall protein translation would shut down to alleviate ER stress by stopping translation of misfolded proteins. Our results highlight that albeit with reduced expression of toxic protein level, neurons still fail to control the homeostasis because of toxic SOD1 aggregated protein.

In Southeastern Chinese SOD1 mutant patients with ALS, the four most frequent mutations in SOD1 gene were p.V48A (4/32, 12.5%), H47R, C112Y, and G148D (3/32, 9.4%). The p.V48A has been reported by another team from Southern China[10], implying that there might be the founder effect existing of p.V48A in Southeastern China. According to a recent nationwide survey on SOD1 in China by Fan's team, p.H47R was the most frequent mutation in China[11] and Japan[12]. In our study, patients with p.H47R and p.C112Y variants presented with the typical SOD1-related phenotype (lower limbs onset and lower motor neuron involvement) and mild disease course (longer diagnostic delay and extended survival time) (Table 1). The most predominant SOD1 gene mutations in total were p.D91A, followed by p.I114T and p.A5V[12]. The mutation p.D91A was absent in our study and absent in Southeastern Chinese demographic, and only one case with p.A5V mutation was found. Therefore, the frequency of specific mutations can vary among different regions. For example, approximately 30% of all Finnish ALS patients have p.D91A mutation and the p.A5V accounts for almost half in SOD1 mutations in the United States[13, 14].

ALS predominance in males was reported by several teams in Mainland and Taiwan in China[11, 15, 16]. However, there was no gender difference in our patients harboring SOD1 mutations. In addition, our results revealed that male SOD1-mutated patients had longer survival time, which is not consistent with the finding by Fan's team[11]. At first, we found that the survival time showed no difference between gender when including all variants (Fig. 4B). Due to the variant p.H47R is associated with long survival time and high frequency in Chinese SOD1-mutated patients, we think it would be a potential bias to affect the statistical analysis. Therefore, we further analyzed the survival time by excluding the p.H47R variant in one male and two female patients, and the results revealed increased survival in male patients (Fig. 4C). Given the variant p.H47R predominance in females in the study conducted by Fan's team[11], this may be the possible reason for this conflicting result. Thus, it deserves our attention when combining p.H47R into the overall results, especially when considering the limited sample size.
One possible explanation for gender differences might be the effect of gonadal hormones. A very recent report demonstrated that dihydrotestosterone (DHT) levels were significantly decreased in all ALS patients, indicating that DHT is probably integral to motor neuron survival(17).

The mean (SD) AAO of the SOD1-mutant patients in this study was 46 ± 11.4 years which is younger than that of the overall Chinese ALS population(18). SOD1-mutant patients were a subgroup in overall ALS population, and similar findings could be seen in Canada(19). The mean (SD) diagnostic delay of SOD1-mutant patients was 10.7 ± 6.2 months, which is shorter than 14 months of overall Chinese ALS patients(20). The SOD1-mutant FALS patients received an earlier diagnosis than the SALS patients (Fig. 3D), which is not consistent with the finding of Fan’s team.

Limitations

Since the SOD1-mutant ALS patients from this cohort all come from Southeastern China, it should be mentioned when using these data because it may only be suitable only Chinese populations. Given the limited sample size, the frequency of 58.9% for FALS probably was overestimated.

Conclusions

Overall, our work provides an updated natural history of SOD1-mutated ALS patients in Southeastern China, which can serve as a supplementary reference database for SOD1-targeted therapy in clinical trials.

Abbreviations

SOD1: Cu/Zn Superoxide dismutase 1; ALS: Amyotrophic lateral sclerosis; WES: Whole exome sequencing; AAO: Age at onset; SD: Standard deviation; FALS: Familial ALS; SALS: Sporadic ALS; ACMG: American College of Medical Genetics and Genomics; VUS: Variant of uncertain significance; ER: Endoplasmic reticulum; UPR: Unfold protein response; DHT: Dihydrotestosterone.

Declarations

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Availability of data and materials

Data can be accessed via email from the corresponding author.

Authors’ contributions

Chen L.X., study concept and design, genetic analysis, interpretation of data, experiments, statistical analysis, and drafting of the manuscript and figures. Xu H.F., Validation of data, experiments. Wang P.S., patient enrollment. Yang X.X., patient enrollment. Wu Z.Y., study concept and design, acquisition of data, study supervision, and critical revision of the manuscript. Li H.F., study concept and design, study supervision, and critical revision of the manuscript. All authors read and approved the manuscript.
Ethics approval and consent to participate

The study was approved by the ethics committee of Second Affiliated Hospital of Zhejiang University School of Medicine and written informed consent was obtained from each subject included in the study.

Consent for publication

Not applicable.

Competing interests

The authors have no competing interests to declare.

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Figures

SOD1 novel variants identified in patients, functional study and SOD1 mutation distribution. a-b Pedigrees of two ALS families carried missense variants of the SOD1 gene. Arrows indicate the proband of the family. c-d Sequence of c.49_50del insCA (p.G17H) and c.400G>T (p.E134*) variants in SOD1 are shown. Arrows indicate variant sites. e-f The p.G17H and p.E134* variants were highly conserved from chimpanzee to zebrafish. g HEK293T cells were transiently transfected with plasmids expressing Vector, p.WT, p.G17H, and p.E134*. The SOD1 protein was detected by anti-Flag antibody. h Quantitative real-time
PCR of SOD1 mRNA levels in HEK293T cells transfected with empty vector, WT, or mutant flag-tagged SOD1 vectors. GAPDH was used as an internal control. The data shown are representative of three independent experiments. Error bars indicate SDs, ***P < 0.001, ****P < 0.0001 (one-way ANOVA). i Immunofluorescence confocal of HEK293T cells expressing WT or mutant SOD1. j Mutation spectrum in SOD1 gene of Southeastern Chinese ALS patients in our ALS cohort.

Figure 2

Age at onset (AAO) of patients with SOD1 mutations. a Plot of rank ordered SOD1-mutant patients showing the median AAO of 47 years. b Plot comparing the AAO among patients carrying mutations in different exons of the SOD1 gene. Patients (n = 24) harboring mutations in exon 1 were older than those (n = 18) harboring mutations in exon 2 (34.6 years vs 51.4 years, p = 0.038). c Plots comparing male and female patients and (d) plots comparing FALS patients with SALS patients; neither comparison identified a significant difference in AAO (p = 0.58 and 0.15, respectively).
Figure 3

Diagnostic delay of patients with SOD1 mutations. a Plot of rank ordered SOD1-mutant patients showing the median diagnostic delay of 9.5 months. b Plot comparing the diagnostic delay showed no differences in different exons of SOD1 gene. c Plots comparing the diagnostic delay exhibited no differences between male and female patients. d plots comparing FALS patients with SALS patients in the diagnostic delay time. neither comparison identified a significant difference in AAO (p = 0.58 and 0.15, respectively). FALS patients (n = 25) received an earlier diagnosis than SALS patients (n = 3) [9.5 (4.8) vs 20.3 (9.3), p = 0.0026].

Figure 4
Survival analysis comparison of patients carrying SOD1 mutations. a Plot of survival probabilities for all patients with SOD1 mutations. The overall median survival time was seen of blue mark (40 months) and the 5-year survival rate was seen of red (30.7%). b Plot of survival probabilities between all male and female patients (median survival 53 months vs 28 months, p = 0.63). c Plot of survival probabilities between male and female after excluding the H47R mutation data, indicating that males had a longer survival time than females (median survival 40 months vs 16 months, p = 0.05).