A novel 10-base pair insertion mutation in exon 5 of the SOD1 gene in a Chinese family with amyotrophic lateral sclerosis

Siyu Chen^a, Mao Li^a, Wenjia Zhu^a, Fengbiao Mao^{b,c}, Jiesi Wang^{b,c}, Zhongsheng Sun^{b,c}, Xusheng Huang^a

^a Department of Neurology, Chinese PLA General Hospital, Beijing 100857, China

^b Beijing Institutes of Life Science Chinese Academy of Science, Beijing 1001101, China

^c University of Chinese Academy of Science, Beijing 10049, China

Corresponding author at: Chinese PLA General Hospital, Beijing, China. Tel.: +86 01055499221;

fax: +86 01055499221; Beijing Institutes of Life Science Chinese Academy of Sciences, Beijing, China.

Tel.: +86 01064864959; fax: +86 01064887229.

E-mail addresses: lewish301@sina.com (X. Huang), zsunusa@yahoo.com (Z. Sun).

Abstract

Amyotrophic lateral sclerosis (ALS) is an adult-onset, progressive, fatal neurodegenerative disease. Several genes are associated with ALS. Copper-zinc superoxide dismutase-1 (*SOD1*) is one of the most commonly mutated genes in ALS, and more than 160 mutations in *SOD1* have been reported. We reported a novel heterozygous insertion mutation that led to a frameshift and a premature termination at position 136 in exon 5 of the *SOD1* gene (c.392_393insGCAAAGGTGG; p.N132Qfs*5) in a Chinese familial ALS pedigree. This mutation in the pedigree demonstrated an autosomal dominant pattern of inheritance and a phenotype characterized by an early onset (approximately 34 years old) with a relatively rapid course (approximately 2 years) and limb onset with respiratory involvement. The clinical feature of the p.N132Qfs*5 mutation was nearly

identical to a previously reported mutation (Gly127insTGGG). Experiments in G127X mice demonstrated that the G127X mutation was pathogenic. *SOD1* activity in the p.N132Qfs*5 mutation carriers in the family decreased significantly compared with normal family members. In conclusion, we identified a novel *SOD1* mutation in an ALS family, which is an important addition to the catalog of *SOD1* mutations in ALS.

Keywords: ALS; SOD1; insertion mutation.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is an adult-onset, progressive, fatal neurodegenerative disease that is primarily caused by the degeneration of motor neurons in the cortex, brain stem, and spinal cord. Approximately 10% of ALS cases are familial (Renton et al., 2014), and the remaining ALS cases are sporadic. The prevalence of ALS is estimated at 4.06-7.89:100,000 (Chio et al., 2013). ALS patients are characterized by progressive paralysis and die from respiratory failure within an average 2-3 years of onset (Renton et al., 2014).

The Copper-zinc superoxide dismutase 1(*SOD1*) gene was firstly reported to be associated with ALS in 1993 (Rosen et al., 1993), and more than 160 mutations in the *SOD1* gene have been reported in the Amyotrophic Lateral Sclerosis Online Genetics database (ALSoD) since then (ALSoD: <http://alsod.iop.kcl.ac.uk/>; Abel et al., 2012). Most mutations are missense mutations, and a few mutations are deletions or insertions. Mutations of this gene account for 12-23% of familial ALS (fALS) and 1-3% of sporadic ALS (sALS) (Chen et al., 2013; Renton et al., 2014 , Chio et al.,

2013). In this study, we identified a novel insertion mutation in the *SOD1* gene in an Chinese ALS pedigree.

2. Case report

The proband (III-3) of a Han Chinese pedigree (Fig. 1A) first noticed progressive weakness of the left forefinger at age 35. Her symptoms spread to involve both upper extremities in the following 6 months with the appearance of atrophy and fasciculation in the left proximal upper arm and hand. Muscle strength of the left proximal and distal upper extremity was Medical Research Council grade 4-5 and grade 2-3, respectively (Great Lakes ALS Study Group, 2003). Tendon reflexes of the upper extremities were decreased, but other reflexes were normal. Hoffmann sign, Babinski signs, and paraspinal muscle reflex were negative. Sensory abnormalities and cognitive impairments were not observed. Electromyography (EMG) demonstrated neurogenic damage of the upper limbs muscles. Cervical magnetic resonance imaging (MRI) revealed no significant abnormalities. Fasciculations spread to her abdomen and her proximal lower extremity approximately 7 months after onset. Target-gene capture and next-generation sequencing of ALS-related genes revealed the existence of a *SOD1* gene mutation. The patient deteriorated approximately 1.5 years after onset. She experienced serious respiratory disability prior to death. A diagnosis of possible ALS was made based on Revised El Escorial criteria (Brooks et al., 2000).

A 33 year old male cousin (III-5) of the proband developed weakness in the right lower extremity. Neurological examination revealed atrophy in the right lower limb and fasciculation in all four extremities. Strength in the right lower extremity was Medical Research Council grade 2-3 and

grade 4-5 in the left lower extremity. Hoffmann sign, Babinski signs, and paraspinal muscle reflex were present. EMG revealed neurogenic damage of the cervical, thoracic, and lumbar segments. Clinically definite ALS was diagnosed based on Revised El Escorial criteria (Brooks et al., 2000). Both of the two patients' mothers (II-2 and II-4) had difficulty in raising head before death at the age of 50.

2.1 Genetic and bioinformatics analyses

Fourteen blood samples were obtained from the pedigree. Genomic DNA was extracted from peripheral blood using a standard procedure. The entire coding regions of *SOD1*, *FUS*, *TARDBP*, *VCP*, *VAPB*, *OPTN*, *UBQLN2*, *ANG*, *SQSTM1*, *CHMP2B*, *ATXN2*, *PFN1*, *DCTN1*, *FIG4*, *ELP3*, *SETX*, *HNRNPA1*, *SPG11*, *NEFH*, *MATR3* genes, of the proband were screened using targeted high-throughput sequencing (Kenna et al., 2013), using primers (Supplementary table 1). The hexanucleotide repeat in *C9orf72* gene was amplified using repeat-primed PCR and methods as previously described (DeJesus-Hernandez et al., 2011). A *SOD1* novel p.N132Qfs*5 mutation (genomic position of chr21:33040818-33040819; NM_000454; c.392_393insGCAAAGGTGG;) was identified in the proband, patient III-5, and 4 other family members using Sanger sequencing. DNA sequences of the 14 family members were confirmed using TA cloning methodology. The insertion mutation (p.N132Qfs*5) of the *SOD1* gene existed in accordance with the Sanger sequence results. The prediction programs PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>), Sorting Intolerant From Tolerant (<http://sift.jcvi.org/>) and Mutation Taster (<http://www.mutationtaster.org>) were used to evaluate the effect of the newly identified mutation in the *SOD1* gene. The Swiss-Model protein structure homology-modeling server

(<http://swissmodel.expasy.org/>; SOD1 Protein Data Bank accession number 4b3e.1.A) was used to examine possible effects of the mutation on the three-dimensional (3-D) structure of the protein.

2.2 SOD1 activities analysis

SOD1 activities were measured in the hemolysates of red blood cells (RBCs) from patient III-5, N132Qfs*5 mutation carriers and wild-type family members using a SOD-typed assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and normalized to hemoglobin (Wang et al., 2014). Because the proband died, *SOD1* activity in her blood was unable to be measured.

3. Results

DNA sequencing analysis indicated a novel heterozygous insertion mutation (c.392_393insGCAAAGGTGG; p.N132Qfs*5) at position 131 in exon 5 of the *SOD1* gene that led to a frameshift mutation, the generation of four new amino acids and a premature stop codon at position 136 in the proband (Fig. 1B), patient III-5, and 4 other asymptomatic members (IV-6, 7, 8, 9) (Fig. 1C) in the family. The 4 asymptomatic members are all under the age of 10. DNA samples of the 8 other adult members in the family were obtained and sequenced. The mutation were not found in their DNA (Fig. 1D), which proved that it segregated with the disease. Codons 130-154 are highly evolutionarily conserved in vertebrates and invertebrates (Fig. 1E). Mutation Taster predicted that this mutation was "disease causing". Therefore, we analyzed this insertion mutation in 345 Han Chinese controls using Sanger sequencing and searched many databases, including 1000 Genome (<http://www.ncbi.nlm.nih.gov/Ftp/>), Single Nucleotide Polymorphism

Database build 137 (<http://www.ncbi.nlm.nih.gov/projects/SNP/>), the Exome Sequencing Project (<http://evs.gs.washington.edu/EVS/>), the Exome Aggregation Consortium (ExAC) database (<http://exac.broadinstitute.org>) and an in-house database containing 2810 normal Chinese Han population exome sequences (Beijing Genomics Institute, Shenzhen, China). No insertion mutation at position 131 was identified in any of these databases, and no mutations in the *C9orf72* or *TARDBP* gene were identified in this pedigree. The crystallographic model predicted by Swiss-Model revealed a significant changes in SOD1 (Fig.1 F). The average *SOD1* activity of patient III-5 and Gly131insGCAAAGGTGG mutation carriers was 58.1% of the wild-type controls (Fig. 1G). The variant N132Qfs*5 will be recorded in the Amyotrophic Lateral Sclerosis Online Database (ALSoD).

4. Discussion

We identified a novel heterozygous insertion mutation (c.392_393insGCAAAGGTGG; p.N132Qfs*5) of the *SOD1* gene in a Chinese fALS pedigree. Unlike other missense mutations of *SOD1* in fALS patients, the mutation in our pedigree involved a 10-base pair (GCAAAGGTGG) insertion at the 131th codon in exon 5 of the *SOD1* gene that caused a frameshift, the generation of four new amino acids (Fig. 1C) and a premature stop codon at position 136. *In silico* analysis and the prediction program Mutation Taster suggested that the p.N132Qfs*5 mutation was the disease-causing mutation that affected features of the protein and altered the splicing site, thus generating a truncated protein. The mutation identified in our patient has not been previously

reported in the Amyotrophic Lateral Sclerosis Online Genetics database (ALSoD) (<http://alsod.iop.kcl.ac.uk>).

Another mutation at the Gly127 position of *SOD1* (Gly127insTGGG) which also caused a frameshift and premature termination (Andersen et al., 1997), has been reported in a Scandinavian fALS case. The pedigree exhibited onset of 37-59 years (mean 48.7 years) in the left extremities and an average duration of 3 years, similar to our pedigree. The transgenic mice model carrying the human mutant Gly127insTGGG *SOD1* gene developed motor neuron degeneration signs with a rapid course (7-10 days) (Jonsson et al., 2004).

Currently, thirteen *SOD1* truncating/frameshift mutations have been found in ALSoD (include 1094 ALS patients) and two *SOD1* truncating/frameshift mutations in ExAC (include 60706 normal people). The frequency of truncating or frameshift mutations in normal people is very low. However, *SOD1* truncating or frameshift mutations account for a certain proportion in ALS patients. This evidence suggests *SOD1* truncation mutations to be associated with ALS.

The p.N132Qfs*5 mutation in the *SOD1* protein lost 19 amino acids that are translated by codons 136-154. However, codons 130-154 in exon 5 of the *SOD1* gene are highly evolutionarily conserved in vertebrates and invertebrates. The crystallographic model revealed that residues 133-136 formed a helix and residues 144-149 and residues 151-152 formed two β -strands in *SOD1*. The helix and the two β -strands formed one rim of the active-site channel of *SOD1* based on the X-ray crystal structure (Richardson et al., 1983). The truncated protein likely dramatically opens the active site channel and increase the catalysis of harmful reaction by simultaneously increasing the accessibility

and flexibility of the metal ions and decreasing the specificity of metal ions (Zu et al., 1997). These structural changes may initiate the mutant *SOD1* protein pathologies in ALS.

Most ALS patients with *SOD1* mutations exhibit reduced enzymatic *SOD1* activities (58% (± 17 ,SD) of normal values) in erythrocytes (Rachele et al., 2013; Saccon et al., 2013). The *SOD1* activity of patient III-5 and p.N132Qfs*5 mutation carriers decreased to 58.1% of normal control subjects in our study. Though a loss of function mechanism is less favored compared with gain of function theory (Julien et al., 2006). A recent study which reviewed two decades of work about mutant *SOD1*, functional data from *SOD1* knockout and transgenic mice, pointed out that loss of function might play a modifying role in ALS (Saccon et al., 2013). Decreased or altered *SOD1* activity underlied the increased oxidative damage, which promoted the generation of monomeric *SOD1*. The monomeric *SOD1* is easier to be misfolded, oligomerized and aggregated, which can cause motor neuron degeneration (Saccon et al., 2013). In a word, loss and gain of function mechanisms potentially coexist in ALS. Further studies are necessary to confirm the certain mechanism of ALS.

Notably, the clinical features of the ALS patients in our pedigree were not identical. The proband exhibited onset in the upper extremities and symptoms of lower motor neuron degeneration. Patient III-5 exhibited onset in the lower extremities and symptoms of both lower and upper motor neuron damage. Variable clinical features in the same pedigree suggest a role for complex inheritance of multiple ALS-related genes (Cady et al., 2015), environmental factors and other factors in ALS pathogenesis in this family. However, 4 asymptomatic p.N132Qfs*5 mutation carriers with normal neurological examinations were noted in this family. The oldest was 10 years old, and the youngest was 2.5 years old. All carriers were younger than the earliest age of onset of 32 years. Patients and

asymptomatic p.N132Qfs*5 mutation carriers require long-term follow-up to identify whether incomplete penetrance and other disease-related factors exist in this family.

Taken together, we described a novel insertion mutation in a Chinese family with an early onset and progressive disease course. This p.N132Qfs*5 mutation is an important addition to the catalog of disease-causing *SOD1* mutations.

Disclosure statement

The authors have no actual or potential conflicts of interest. The appropriate approval and procedures were used for all human subjects. This work was approved by the ethics committee of the Beijing Institute of Life Science, Chinese Academy of Science. Informed consent was obtained from all human subjects or their parents. This work was supported by a grant of the National Science Foundation of China (No.81441042).

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Figure legend

Fig. 1. A novel p.N132Qfs*5 mutation in the Cu-Zn superoxide dismutase 1 (*SOD1*) gene identified in a Chinese amyotrophic lateral sclerosis (ALS) family. (A) Pedigree of the ALS family. Square indicates male; circle, female; diagonal, deceased; filled symbol, affected member; asterisk, DNA available; a black circle in a square or circle, asymptomatic carrier; arrow, proband. (B) A heterozygous insertion mutation identified using Sanger sequencing in the proband (III-3) with a GCAAAGGTGG insertion at the 131th codon in exon 5 of the *SOD1* gene. (C) A heterozygous insertion mutation was also identified in the asymptomatic patient IV-6 with a GCAAAGGTGG insertion at the 131th codon in exon 5 of the *SOD1* gene. (D) Sanger sequence of the 127th –to 154th codons in exon 5 of the *SOD1* gene from the normal control. (E) Multiple sequence alignment translated by the 109th to 154th codons of *SOD1*. This region is highly conserved among species. (F) Three-dimensional structures of the *SOD1* protein wild-type and p.N132Qfs*5 mutation. (G) *SOD1* activity normalized to hemoglobin (Hb). The average level of *SOD1* activity in p.N132Qfs*5 mutation carriers was 58.1% of the wild-type (WT) controls.

B Normal IV-5

L G K G G N E E S T
 T T G G G C A A G G T G G A A T G A G A A G T A C A

C Proband

L G K G G N E E S T
 T T G G G C A A G G T G G A A T G A G A A G T A C A

C A A G G T G G A A T G G A
 Q R W K

D Highly conserved amino acid at condon 109-154

<i>H. sapiens</i>	109	GDNCIIIGATLVVKKASDCLRG--GHESTSTGNAGRLACGVIGIAQ-	154
<i>P. troglodytes</i>	109	GDNCIIIGATLVVKKASDCLRG--GHESTSTGNAGRLACGVIGIAQ-	154
<i>M. musculus</i>	109	GESEIIIGATLVVKKASDCLRG--GHESTSTGNAGRLACGVIGIAQ-	154
<i>M. m. domestica</i>	109	GDNCIIIGATLVVKKASDCLRG--GHESTSTGNAGRLACGVIGIAQ-	154
<i>R. norvegicus</i>	109	GESEIIIGATLVVKKASDCLRG--GHESTSTGNAGRLACGVIGIAQ-	154
<i>D. rerio</i>	109	GDNCIIIGATLVVKKASDCLRG--GHESTSTGNAGRLACGVIGIAQ-	154
<i>C. lupus</i>	109	GDNCIIIGATLVVKKASDCLRG--GHESTSTGNAGRLACGVIGIAQ-	154
<i>B. taurus</i>	107	GESEIIIGATLVVKKASDCLRG--GHESTSTGNAGRLACGVIGIAQ-	152
<i>G. gallus</i>	108	GDNCIIIGATLVVKKASDCLRG--GHESTSTGNAGRLACGVIGIAQ-	154