CHARACTERISTICS OF SOD1 GENE MUTATION IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS AND ITS RELATIONSHIP WITH CLINICAL PHENOTYPE

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ABSTRACT

Objective*: To analyse the type of copper-zinc superoxide dismutase (SOD1) gene mutation and its relationship with clinical phenotype in patients with amyotrophic lateral sclerosis (ALS).*

Methods*: Twenty-seven patients with ALS (probands of familial ALS) diagnosed and treated in the Department of Neurology of our hospital from 2015 to 2019 were selected for this study. The clinical data and peripheral blood samples of all patients were collected, and five exons of the SOD1 gene on 27 probands were detected via the real-time quantitative PCR method and direct sequencing method to analyse their relationships with the clinical phenotype.*

Results*: The PCR method was used to detect five exons of the SOD1 gene in 38 patients. The results showed that three heterozygous missense mutations were detected in 6 probands, and the detection rate of SOD1 positive mutations was 22.22%. There were 13 patients with the p.His46Arg (C:140A >G) mutation in their families, and all of them had unilateral distal lower extremity onset in which the lower motor neuron lesion was dominant, the F1-III8 reflex was active, the survival time of the F1 and XX probands were 18 and 20 years, respectively, and the survival time of patients in the F2 and F3 families reached 20 years. Three cases in the F4 family had lower extremity onset, with F4-III5 producing lower extremity proximal onset and upper and lower motor neuron damage. The F5-II8 cases in the F5 family showed lower motor neuron damage, and the survival time of patients was 3 to 4 years. The F6-III1 case showed weakness at the distal end of the unilateral upper limb, but the symptoms were mild and accompanied by mild atrophy of the small muscles of the hand and tingling pain in the hand. An Electromyogram (EMG) indicated extensive neurogenic damage and slow progression of the disease.*

Conclusion*: SOD1 gene mutations still dominate in familial ALS patients, with most SOD1 mutations being associated with clinical phenotypes, and p.His46Arg (C:140A>G) mutations having characteristic clinical phenotypes.*

Keywords: *amyotrophic lateral sclerosis (ALS), copper-zinc superoxide dismutase (SOD1), genic mutation, clinical phenotypes.*

DOI: 10.19193/0393-6384_2021_2_124

Received *August 15, 2020;* ***Accepted*** *January 20, 2021*

Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that involves mainly upper and lower motor neurons. ALS cases can be divided into familial and sporadic cases, in which sporadic cases account for 5% to 10% of total cases, and there are no differences in clinical manifestations between the two types[(1)]. The clinical manifestations in ALS patients are mostly inability to speak, dysphagia, progressive myasthenia with muscular atrophy and respiratory failure. Most patients die of respiratory failure within 3 to 10 years after the onset of ALS, which makes clinical treatment very difficult. Familial ALS is mainly divided into ALS1~8, ALS-10 and other subtypes, among which the most studied is ALS-1. The copper-zinc superoxide dismutase (SOD1) gene is the main ALS pathogenic gene, and it has been confirmed that this gene is closely related to the pathogenesis in 20% of the cases of familial ALS and 5% of the cases of sporadic ALS[(2-3)]. SOD1 genes are mainly located in 21q22, including five

exons and four introns that are mainly distributed in the cytoplasm and nucleus of eukaryotic cells, and can also exist in the mitochondrial membrane space and the periplasmic space of bacteria. The SOD1 gene has important antioxidant effects and can prevent oxidative damage in the body[4]. The pathogenesis of ALS is not clear at present, although some scholars believe that the mutations in the SOD1 gene can reduce superoxide dismutase activity, leading to the decreasing ability to scavenge superoxide in vivo and oxidative damage to the body[5]. SOD1 mutations are mainly concentrated in ALS patients in Europe and America. Hence, this study aimed to analyse the SOD1 gene mutation types and their relationships with clinical phenotypes in patients with ALS in China.

Data and methods

Research subjects

Twenty-seven patients with ALS (probands of familial ALS) diagnosed and treated in the Department of Neurology of our hospital from 2015 to 2019 were selected for this study. The inclusion criteria were based on the diagnostic criteria for ALS established by the World Union of Neurology[6]. All patients had upper and lower neuron lesions. The disease had developed progressively in all patients, and patients had one or more sites of muscle bundle tremors and conduction blocks. Patients and their families provided informed consent and cooperated with the treatment. This study was approved by the Hospital Ethics Committee. The exclusion criteria were sensory dysfunction, autonomic nerve dysfunction, incomplete clinical data and symptoms and signs of extrapyramidal diseases.

Experimental reagents and instruments

The genomic DNA extraction kit was purchased from the Beijing Tiangen company, and the SOD1 primers were synthesized by Shanghai Yingjun Biological. The PCR reaction reagent was purchased from Dalian TaKaRa Bioengineering Co., Ltd., while the TAQ enzyme kit was purchased from TOYOBO A112-01, Japan. The agarose was purchased from Shanghai Boya Biotechnology Co., Ltd.

The PCR instrument was purchased from ABI (USA), while the centrifugal precipitator was purchased from the Shanghai Surgical Instrument factory. A high-speed table-top centrifuge was purchased from the Shanghai Anting Scientific Instrument factory, while a 3730 automatic sequencer was purchased from Applied Biosystems (USA). The imager was purchased from Bio-Rad (USA), and the horizontal electrophoresis tank was purchased from the Beijing 61 Instrument factory.

SOD1 exon detection

DNA extraction and purification: 2 ml of venous blood collected from all patients were added to test tubes containing ACD anticoagulant. After mixing well, 3 ml of red blood cell lysis buffer was added to each test tube and incubated at room temperature for 10 min, then the white blood cells were precipitated by centrifugation after blood transparency was reached. The supernatant was discarded and 100μl of residual fluid was left, which was then mixed well to obtain suspended white blood cells. The cells were transferred into new EP tubes after centrifugation, the white blood cells were precipitated and the supernatant was discarded. White blood cells were resuspended with 200μl PBS buffer, 200μl of detergent and 200μl of protease were added and mixed in well, then the solution wax incubated for 10 min. Next, 200μl of anhydrous ethanol was added to each EP tube and mixed well, then the tube was placed in the Tiangen spin column. After centrifugation, the supernatant was discarded and the liquid in the tube was collected. Then 500μl of extraction solution was added to the column once more, and it was centrifuged to empty the liquid into the collection tube. Afterwards, 700μl of IPW washing solution was added to the Tiangen spin column and centrifuged, then 500μl of IPW washing solution was added again for further centrifugation. The column was added to 150μl of AE and left standing for 5 min. After centrifugation and collection, the DNA was purified. Next, 5μl of l×loading buffer was added to the purified DNA, then the solution was mixed well for electrophoresis. Using the position of the indicator during electrophoresis, the position of the electrophoretic band was observed via the imager, then pictures were taken. Finally, the size of the purified DNA was determined.

Primer design: A PCR assay was used to amplify five exons of the SOD1 gene. The amplification system consisted of 1μl of DNA, 0.5μl of LaqTMDNA polymerase, 0.5μl each of upstream and downstream primers, 0.5μl of dNTP mixture and 12.5μl of 2×GC Buffer I. Hence, the amplification sequence covered the full-length coding region and the interfaces between introns and exons.

Amplification of target fragments by PCR: PCR amplification was carried out using the TAQ enzyme

kit following standard procedures and the PCR automatic amplification instrument. The dry powder was centrifuged for a few seconds, then the appropriate amount of RNase-free H2O was added according to the instructions. The working solution concentration of the primer was 10 μmol/L. In particular, 7.2μl of RNase-free H2O, 10μl of 2×tap Master Mix, 0.4μl of upstream primer, 0.4μl of downstream primer and 2μl of genome DNA were added to the 0.2 mL PCR tube in turn, then pre-denatured at 94°C for one and a half minutes, denatured at 94°C for a half a minute, annealed at 55 to 61°C for a half a minute and extended at 72°C for one minute for a total of 30 cycles, then extended at 72°C for five minutes and cooled down to 4°C. The PCR products were sequenced in a 3730 DNA automatic sequencer.

Results

Basic clinical data for ALS patients

In this study, all of the 27 familial ALS patients were from mainland China and presented with autosomal-dominant hereditary disease. Among them, there were 14 males and 13 females with ages of onset ranging from 18 to 68 years old and a mean age of 47.58±10.79 years old. There were 25 cases with initial symptoms at the spinal cord, and two cases originating at the medulla oblongata. All patients were examined by electromyography, and the results showed that acute and chronic neurogenic lesions were distributed in the brainstem, cervical, thoracic and lumbar segments.

PCR detection of SOD1 gene mutation

The PCR method was used to detect five exons of the SOD1 gene in 38 patients. Three kinds of heterozygous missense mutations were detected in six probands, and the detection rate for SOD1 positive mutations was 22.22%. The data are shown in table 1.

Three kinds of heterozygous missense mutations were detected in six probands, and three cases with the heterozygous missense mutations located in exon 2 had carried mutations for which the cDNA base at site 140 was mutated from A to G and the amino acid site 46 was mutated from histidine to arginine: p.His46Arg (C:140A>G). The mutation sequencing map for these cases is shown in figure 1.

Two cases of heterozygous missense mutations located on exon 2 carried by probands were found for which the cDNA base at site 143 was mutated from T to C and the amino acid at site 47 was mutated from valine to alanine: p.Val47Ala (C:143T>C). The mutation sequencing map for these cases is shown in figure 2.

Proband	Sex	Age of onset	Course of disease (years)	Site of onset	Clinical type	Segments involved in electromyography	Number of patients in the family	Mutant exon	Nucleotide alterations	Amino acid changes
F1-III8	Female	45	10	Distal lower limbs	Lower motor neuron	Cervical + lumbar	5	2	C:140A>G	p.His46Arg
F2-III1	Male	48	2	Distal lower limbs	Lower motor neuron	Cervical + lumbar	5	2	C:140A>G	p.His46Arg
F3-II5	Female	56	0.67	Distal lower limbs	Lower motor neuron	Cervical + lumbar	3	2	C:140A>G	p.His46Arg
F4-II5	Female	53	1	Lower limbs	Lower and upper motor neuron	Cervical + lumbar +thoracic	3	2	C:143T>C	p.Val47Ala
F5-II8	Female	43	1	Lower limbs	Lower motor neuron	Cervical + lumbar +thoracic	5	2	C:143T>C	p.Val47Ala
F6-III1	Male	39	2	Upper limb	Lower and upper motor neuron	Cervical + lumbar + thoracic + brainstem	2	2	C:112G>C	p.Gly37 Arg

Table 1: Clinical features and mutation sites on the SOD1 gene.

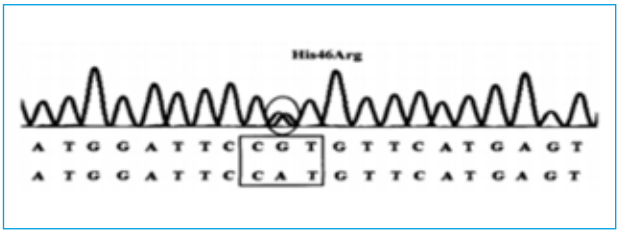

Figure 1: P.His46Arg (C:140A>G) mutation sequencing map for exon 2.

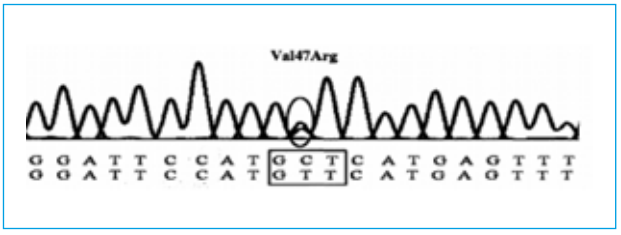

Figure 2: P.Val47Ala (C:143T>C) mutation sequencing map for exon 2.

One case of the heterozygous missense mutations located on exon 2 involving the proband showed the cDNA base at site 112 mutated from G to C and the amino acid at site 37 mutated from glycine to arginine: p.Gly37 Arg (C:112G>C). The mutation sequencing map for this case is shown in figure 3.

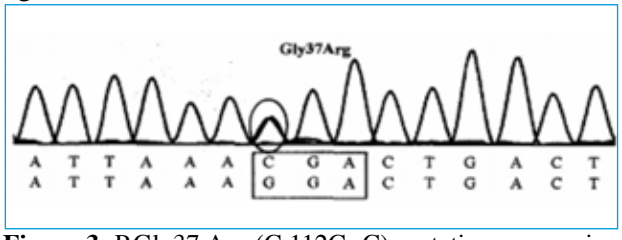

Figure 3: P.Gly37 Arg (C:112G>C) mutation sequencing map for exon 2.

Characteristics of clinical manifestations of SOD1 positive mutations

Pedigree diagrams of three p.His46Arg (C:140A>G) mutations

There were 13 patients with the p.His46Arg (C:140A >G) mutation in the family, all of which displayed unilateral distal lower extremity onset in which the lower motor neuron lesion was dominant, the F1-III8 reflex was active, and the survival time of the F1 proband was 18 years and 20 years, respectively, and the survival time of patients in F2 and F3 families reached 20 years. The pedigree diagram is shown in figure 4.

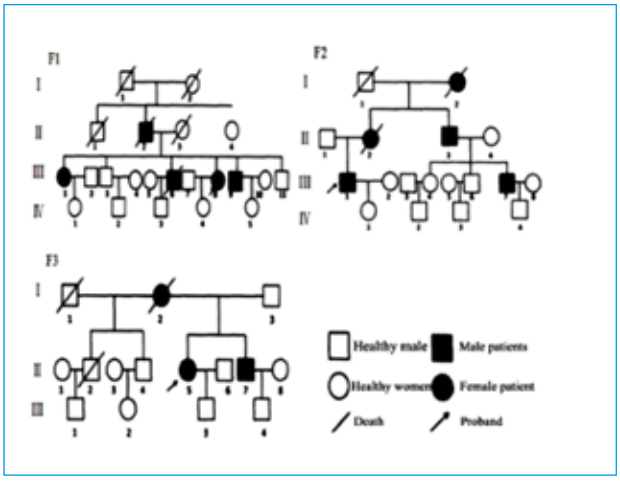

Figure 4: Pedigree diagram of the p.His46Arg (C:140A>G) mutation.

Pedigree diagrams for two p.Val47Ala (C:143T>C) mutations

Three cases in the F4 family involved lower extremity onset, with the F4-III5 case having lower extremity proximal onset and upper and lower motor neurons damage. The F5-II8 cases in the F5 family showed lower motor neuron damage, and the survival time was 3 to 4 years. The pedigree diagrams for these two p.Val47Ala (C:143T>C) mutations are shown in figure 5.

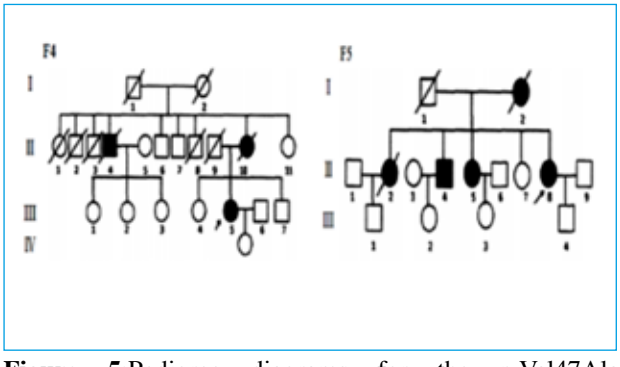

Figure 5:Pedigree diagrams for the p.Val47Ala (C:143T>C) mutations.

Pedigree diagram of one p.Gly37 Arg (C:112G>C) mutation

The F6-III1 case exhibited weakness at the distal end of the unilateral upper limb, but the symptoms were mild and accompanied by mild atrophy of the small muscles of the hand and tingling pain of the hand. An electromyogram (EMG) indicated extensive neurogenic damage and slow progression of the disease. The pedigree diagram is shown in figure 6.

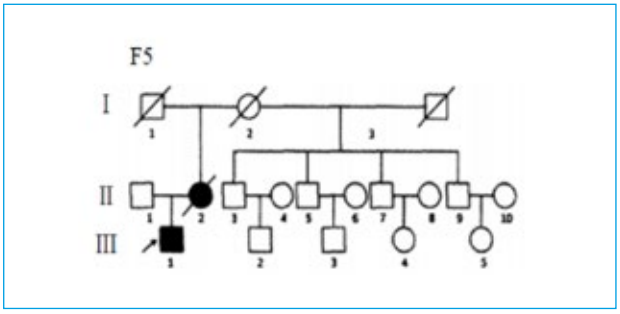

Figure 6: Pedigree diagram for the p.Gly37 Arg (C:-112G>C) mutation.

Discussion

ALS is an adult-onset neurodegenerative disease with chronic progressive development. In the later stage of the disease, patients often show severe muscular atrophy and bulbar paralysis, generally without sensory disorders and defecation disorders, and will gradually develop dysphagia and quickly progress to respiratory failure.

At present, ALS pathogenesis is not clear, and there is no particularly effective drug treatment[7]. Relevant data show that ALS exhibits selective apoptosis in the brain and spinal cord and inclusion bodies formed by abnormal protein deposition, which can provide the basis for its clinical treatment[8]. ALS-1 and the SOD1 gene mutations have been most extensively researched in terms of familial ALS. There is a consensus that SOD1 gene mutations can lead to the acquisition of the protein toxicity function. Recently, some scholars have found that reducing the expression of SOD1 genes in astrocytes can delay the progression of the disease significantly[9].

SOD1 is an antioxidant protein that was discovered in 1973 and has about 150 different mutation sites. Further, it is found in large amounts in nerve tissue, the liver, and red blood cells, and is the largest subgroup of fALSs. Its mechanism has been determined to be the amplification of toxic protein function rather than the loss of enzyme activity. Due to the defects in the glutamate transporter near the

synapse of the glial cells, the released neurotransmitter glutamate cannot be removed in time, leading to toxic effects[10]. SOD1 can exist inside and outside of cells and uses different ways to increase the production and release of oxygen free radicals and make motor neurons sensitive to glutamate poisoning, further aggravating neurotoxicity[11]. Neurofilaments play an important role in axonal growth, and abnormal aggregations of neurofilaments have been found in motor neurons with a SOD1 mutation. Some scholars have found that the slowdown in axonal transport speed can occur before SOD1 transgenic symptoms, which has a protective effect on motor neurons[12].

At present, more than 150 types of SOD1 mutations have been found and reported internationally, most of which are missense mutations. The detection rate of the SOD1 gene was different in patients with familial ALS compared to other population groups. Some scholars conducted SOD1 gene detection in 15 Han families and found that about 20% of the families carried a SOD1 gene mutation[13]. The age of onset, clinical features, pathological features, and survival time were different for different SOD1 mutation genotypes. For example, the common A47V mutation in the North American population appears to trigger the reduced involvement of upper motor neurons and shorter survival times[14].

In this study, the PCR method was used to investigate five exons of the SOD1 gene in 38 patients. The results showed that three heterozygous missense mutations were detected in 6 probands, and the detection rate of SOD1 positive mutations was 22.22%, which is consistent with the research of Chen Yan, Dong Yi, and other scholars[15]. Among the clinical phenotypes of SOD1 mutations in this study, there were 13 patients with the p.His46Arg (C:140A >G) mutation in their families, all of whom exhibited unilateral distal lower extremity onset, a dominant lower motor neuron lesion, and an active F1-III8 reflex. The survival time for the F1 proband was 18 years and 20 years, respectively, and the survival time of patients in F2 and F3 families reached 20 years. It is suggested that this type of mutation dictates a benign course of the disease and no obvious damage to upper motor neurons. Three cases in the F4 family with the 2 p.Val47Ala (C :143 T >C) mutation exhibited a lower extremity onset, with the F4-III5 case exhibiting lower extremity proximal onset and upper and lower motor neurons damage. The F5-II8 cases from the F5 family showed lower motor neuron damage and survival times of 3 to 4 years. It is suggested that the disease progress in this type of patient is rapid with diverse clinical manifestations. The F6-III1 case with the p.Gly37Arg (C :112G >C) mutation showed weakness at the distal end of the unilateral upper limb, but the symptoms were mild and accompanied by mild atrophy of the small muscles of the hand and tingling pain of the hand. An electromyogram (EMG) indicated extensive neurogenic damage and slow progression of the disease.

In conclusion, SOD1 gene mutations still occupy first place in patients with familial ALS, as most SOD1 mutations are associated with clinical phenotypes, and the clinical phenotypes of p.His46Arg (C:140A>G) mutations are characteristic of the disease.

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ACKNOWLEDGEMENT
This paper was supported by the science and technology plan of Chengguan District, Lanzhou City for detecting the correlation between SOD1 gene polymorphism and clinical manifestations in ALS patients in Gansu Province (No. 2018kjgg0032).

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