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An Iranian familial amyotrophic lateral sclerosis pedigree with p.Val48Phe causing mutation in *SOD1*: a genetic and clinical report

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ABSTRACT

Objective(s): Amyotrophic lateral sclerosis (ALS), a fatal progressive neurodegenerative disorder, is the most common motor neuron disease in European populations. Approximately 10% of ALS cases are familial (FALS) and the other patients are considered as sporadic ALS (SALS). Among many ALS causing genes that have been identified, mutations in *SOD1* and *C9orf72* are the most common genetic causes of the disease. In Iranian patients, it has been shown that *SOD1*, as compared to *C9orf72*, plays a much more prominent role. To date, more than 170 mutations have been reported in *SOD1*. Genotype/phenotype correlation with respect to either different causative genes or different mutations of a specific gene has not been well established.
Materials and Methods: Five exons of *SOD1* and flanking intronic sequences of an Iranian FALS proband were screened for mutations by direct sequencing. Also, the clinical features of the proband were described.
Results: Heterozygous *p.Val48Phe* causing mutation was identified in *SOD1*. Age at onset was 29 years and site of the first presentation was the lower extremity in the proband.
Conclusion: The *p.Val48Phe* causing mutation appears to cause early onset of ALS.

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Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterized by dysfunction and degeneration of both upper motor neurons (UMNs) in the cortex and lower motor neurons (LMNs) in the brainstem and spinal cord that leads to muscular paralysis and ultimately death (1–3). It is the most common motor neuron disease in European countries (4, 5). Its incidence and prevalence in these countries are 1-2/100,000/year and 4-13/100,000/year, respectively (6, 7). Its clinical features depend on several factors, including age at onset of symptoms (one to 94 years) (8, 9), site of onset of symptoms (limbs or bulbar) (10), rate of progression (11, 12), and survival time (few months to over 10 years) (6, 10) . The ultimate cause of death in ALS patients is usually respiratory failure.

Genetics is the source of at least a part of the variability associated with ALS. The majority of patients are sporadic (SALS), while 1-13% of cases in different epidemiological studies were reported to have more than one affected individual in their families and such cases are known as familial ALS (FALS) (13). The mode of inheritance in FALS families is usually autosomal dominant. Mean age at onset of FALS patients is approximately 10 years lower than SALS patients, but they are clinically indistinguishable (9). To date, at least 19 ALS causing genes have been identified (http://alsod.iop.kcl.ac.uk/) (14-16) and approximately, 50–60% of FALS patients carry mutations in these genes (6). Mutations in *SOD1* and *C9orf72* are the most common causes of disease, although their relative contributions vary in different populations (11, 17–24). *SOD1* and *C9orf72* are, respectively, the first and one of the most recently identified ALS genes (17, 18, 24). Recently, we showed that mutations in *SOD1* are more common than mutations in *C9orf72* among Iranian ALS patients (11, 21).

Here, we describe the clinical features of an Iranian FALS patient who harbors a mutation in *SOD1* that causes p.Val48Phe. Before, the mutation was once reported in an Italian patient, but detailed clinical

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features of the patient were not presented (25).

Materials and Methods

This project was performed in accordance with the Helsinki Declaration and approved by the ethics board of the University of Tehran, Tehran, Iran. All participants or their responsible guardians consented to participate after being informed about the project.

Based on El Escorial criteria (26), the proband was diagnosed as definite ALS by a neurologist (SN) in Neuromuscular Clinic of Shariati Hospital, Tehran, Iran. He belonged to a large FALS pedigree that in addition to the proband, includes six ALS patients from four generations (Figure 1). DNA of the proband was isolated according to the standard phenol-chloroform method. Five exons of *SOD1* were amplified by polymerase chain reaction (PCR) (Supplementary Table 1, 2) (11). The sequences of all primers that were used are available upon request. All PCR products were sequenced with the same primers that were used in the PCRs, using the ABI big dye chemistry and an ABI Prism 3700 instrument (Applied Biosystems, Foster City, CA). *SOD1* reference sequences were NC_000021.8, NM_000454.4, and NP_000445.1. Upon identification of the c.142G>T variation that affects p.Val48Phe in the encoded protein, the variation was screened in 100 Iranian control individuals who were over 60 years old using an allele specific PCR protocol. To assess conservation of p.Val48, amino acid sequences of SOD1 proteins from 17 species were obtained from Uniprot; http://www.uniprot.org/uniprot/ and aligned using ClustalW2 software; http://www.ebi.ac.uk/Tools/msa/clustalw2/.

Additionally, the SIFT; http:// blocks.fhcrc. org/sift/SIFT.html, PolyPhen; http://genetics.bwh.harvard.edu/pph2, Panther; http://www.pantherdb.org/tools/csnpScoreForm.jsp and SNAP; https://rostlab.org/services/snap/submit bioinformatics tools were used to predict the potential pathological effects of p.Val48Phe on the encoded protein.

Results

Genetic analysis

In the encoded SOD1 protein, c.142G>T variation that causes p.Val48Phe was observed in the heterozygous state in the DNA of the proband (Figure 2). No additional variation was detected. The only surviving affected individual in the pedigree (III-1) lives in Europe and was not available for genetic analysis. Furthermore, segregation analysis in the pedigree was not possible because none of the unaffected members of the pedigree consented to genetic analysis; they did not want to know whether or not they carried the mutated allele. However, the c.142G>T variation was not observed in 100 unrelated healthy elderly Iranian control individuals. Furthermore, valine at positions corresponding to p.48 in the human SOD1 protein is well conserved across species from Caenorhabditis elegans to Homo sapiens (Table 1). The SIFT, PolyPhen, Panther and SNAP tools predicted, respectively, that the substitution is damaging, probably damaging, deleterious, non-neutral. Before, the same variation was once reported as the cause of ALS in an Italian ALS family (25). All togethS1er, our data led us to conclude that the p.Val48Phe causing variation in *SOD1* was the probable cause of ALS in the proband and his affected relatives. The inheritance pattern of ALS in the pedigree suggests an autosomal dominant mode of inheritance, consistent with observation of a single mutated allele in the proband (Figure 1).

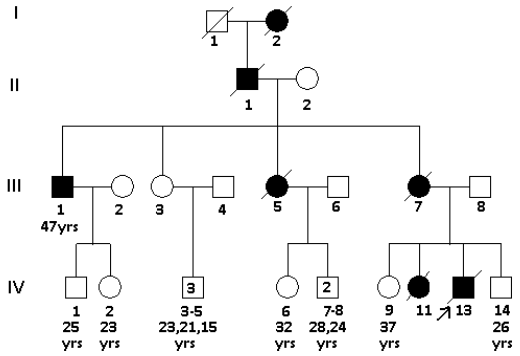

Figure 1. ALS164 pedigree. ■ and ●, ALS affected individuals; □ and ○, asymptomatic individuals; arrow shows proband; present age for some individuals is shown

Clinical data

The ALS patient who was studied here is a member of a FALS pedigree (ALS164) that includes seven ALS-diagnosed patients. The male:female ratio of the patients is 3:4. Five patients had died before the start of the study, and now the proband is also deceased. Available clinical information on five affected members of the pedigree belonging to generations III and IV is presented in Table 2. The average of age at onset of symptoms was 34.6 years (range: 29-45 years). Four patients died 2.5 to 3 years following the onset of symptoms, and the

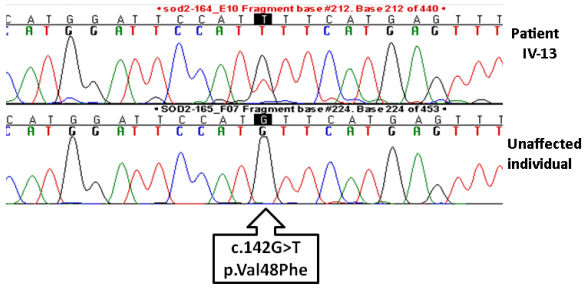

Figure 2. DNA sequence chromatograms showing the C.142G>T mutation and the wild type sequence. The mutation that causes p.Val48Phe is evident in the in the heterozygous state in the chromatogram of the proband

Table 1. Conservation of p.Val48 in SOD1 proteins

Organism	Seq ID*	Amino acid sequence**
Homo sapiens	P00441	TEGLHGFH**V**HEFGDNTA
Pan troglodytes	P60052	TEGLHGFH**V**HEFGDNTA
Macaca mulatta	Q8HXQ0	TEGLHGFH**V**HQFGDNTQ
Bos taurus	P00442	TEGDHGFH**V**HQFGDNTQ
Equus caballus	P00443	TKGDHGFH**V**HEFGDNTQ
Cavia porcellus	P33431	VEGKHGFH**V**HEFGDNTQ
Sus scrofa	P04178	AEGDHGFH**V**HQFGDNTQ
Ovis aries	P09670	TEGDHGFH**V**HQFGDNTQ
Canis familiaris	Q8WNN6	TEGEHGFH**V**HQFEDXTQ
Oryctolagus cuniculus	P09212	TEGLHEFH**V**HQFGDNRQ
Rattus norvegicus	P07632	TEGEHGFH**V**HQYGDNTQ
Mus musculus	P08228	TEGQHGFH**V**HQYGDNTQ
Gallus gallus	P80566	SDGDHGFH**V**HEFGDNTN
Lampanyctus crocodilus	P81036	APGLHGFH**V**HAFGDNTN
Prionace glauca	P11418	TPGKHGFH**V**HAFGDNTN
Xiphias gladius	P03946	TPGEHGFH**V**HGFGDNTN
Caenorhabditis elegans	P34697	TPGLHGFH**V**HQYGDSTN

earliest presentations involved the limbs in these individuals. Presentation in the fifth patient (III-1) was bulbar; age at onset for this individual was approximately 13 years more than the average age at onset of the other patients. Patient III-1 is now, two years after the onset, in the final stages of disease. He breathes with the help of a ventilator and is completely paralyzed, unable to speak and swallow. More detailed clinical data on the proband is presented below.

The proband was a 31-year-old man (IV-13, Figure 1) who presented with a two year history of weakness and atrophy of the limbs which had been started in the left hand and gradually progressed sequentially to involve the right leg, the right hand and the left leg. He mentioned that there was muscle twitching at the beginning, but it was disappeared after a few months. He had no sensory complaint or sphincter dysfunction. Past medical history was unremarkable.

Neurological examination showed normal mental state, tongue atrophy and fasciculation and wasting of left upper extremity. Asymmetric quadriparesis which was more severe in left upper and right lower extremities was seen. Biceps and triceps reflexes were increased on the right side, and were absent on the left side. Right knee and ankle jerks were also absent. Plantar reflexes were downward and sensory examination was intact.

Electromyography that was performed two years after onset of symptoms, showed denervation, fasciculation and reinnervation in various muscles innervated by cranial, cervical, thoracic and lumbar segments. Sensory potentials were normal. The findings were interpreted as definite motor neuron disease according to Awaji criteria (27). Laboratory studies were normal, as were results of brain and cervical spine magnetic resonance imaging (MRI).

Riluzole 50 mg two times a day was started. The patient had progressive deterioration.

Table 2. Clinical features amyotrophic lateral sclerosis patients of pedigree ALS164

Individual ID	Sex	Age at onset (years)	Present age (years)	Age at death (years)	Disease duration (years)	Site of onset
I-2	F	?	Dead	?	?	?
II-1	M	?	Dead	?	?	?
III-1	M	45	47	Alive	_	Bulbar
III-5	F	32	Dead	35	3	Lower extremity
III-7	F	35	Dead	37.5	2.5	Lower extremity
IV-11	F	32	Dead	35	3	Upper extremity
IV-13	M	29	Dead	32	3	Upper extremity

F: female; M: male

He voluntarily entered a clinical trial of autologous mesenchymal stem cell transplantation with intraspinal injection (IRCT201107221696N3). Repeated electro-myography-nerve conduction velocity (EMG-NCV) performed prior to transplantation evidenced reduced compound muscle action potential (CMAP) motor amplitude as compared to his first electromyography. Three months after transplantation, his pulmonary function test showed a forced vital capacity of 75%. The patient remained in a stable and good condition during a four months follow-up. Then, one night he developed severe dyspnea, was admitted to the hospital with a possible diagnosis of pulmonary emboli, and died a few hours later. The details of the transplantation protocol are not presented here.

Discussion

SOD1 encodes copper-zinc superoxide dismutase, which is an evolutionarily highly conserved enzyme that catalyzes the conversion of toxic superoxide anion to hydrogen peroxide and molecular oxygen. In various studies, mutations in *SOD1* were observed in 12-23% of FALS patients (average: 20%) and in 0 to 7 percent of SALS patients (average: 3%) (13, 15, 28). In Iranian ALS patients, these mutations were found in 38.5% of the FALS probands, and 4.25% of the SALS cases (11,12). Over 170 different *SOD1* mutations have been reported so far (11, 14, 21, 22). While it has been generally difficult to establish clear genotype- phenotype correlations for specific mutations and even for different causative genes, a few exceptions exist. Mutations in *SOD1* that cause p.Asp90Ala and p.Leu144Ser are associated with long survival time (11, 29), while p.Ala4Val and p.Gly85Ser are associated with rapid progression of disease (30).

In the present study, we described an Iranian FALS pedigree. The proband of the pedigree harbored a mutation in *SOD1* that causes p.Val48Phe in the encoded protein. ALS inheritance in the pedigree was autosomal dominant, without evidence of anticipation. Before, the p.Val48Phe mutation was once reported in an Italian ALS family (25). Although detailed clinical findings were not presented in the earlier finding, age at onset of symptoms in the proband was reported to be about 36 years. This is close to age at onset in patients of ALS164 pedigree (average: 34.6 years). The father of the Italian proband had died from ALS at the age of 39. Therefore, it seems that the p.Val48Phe mutation causes early onset of disease. Age at onset, survival time and limb onset presentation were notably uniform among four of five patients in the ALS164 pedigree. The fifth patient (III-1) differed from the others and had a much higher age at onset and a bulbar presentation. These observations provide evidence that the p.Val48Phe mutation can result in different clinical features even within a single pedigree. The efficacy of the autologous mesenchymal stem cell transplantation in the proband could not be assessed.

Conclusion

As stated above, a clear genotype-phenotype correlation exists for only a few ALS causing *SOD1* mutations. Based on clinical data on the Iranian family which was described here and the available data on a previously reported Italian ALS patient who harbored the same mutation, it appears that p.Val48Phe causing mutation in *SOD1* mutation causes ALS with an early onset. This having been said, there was some variability in just how early symptoms manifested. Age at onset of four out of five patients ranged between 29 and 36 years while it was notably higher (45 years) for one of them. Limb onset presentation was a common feature among four out of five Iranian patients, but onset was bulbar in the patient who had showed the latest onset. It can be concluded that while the window of clinical presentation for the p.Val48Phe mutation is relatively narrow, particularly with respect to age at onset, it is not strictly uniform.

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References

1. Nelson LM. Epidemiology of ALS. Clin Neurosci 1996; 3:327–331.
2. Charcot JM, Joffroy A. Deux cas d'atrophie musculaire progressive avec lesions de la substance grise et des faisceaux antero-lateraux de la moelle epiniere. Arch Physiol Neurol Pathol 1869; 2:744.
3. Rowland L, Shneider N. Amyotrophic lateral sclerosis. N Engl J Med 2001; 344:1688–16700.
4.Hirtz D, Thurman DJ, Gwinn-Hardy K, Mohamed M, Chaudhuri a R, Zalutsky R. How common are the "common" neurologic disorders? Neurology 2007; 68:326–337.
5. Rison R a, Beydoun SR. Amyotrophic lateral sclerosis-motor neuron disease, monoclonal gammopathy, hyperparathyroidism, and B12 deficiency: case report and review of the literature. J Med Case Pep 2010; 4:298.
6. Sabatelli M, Conte a, Zollino M. Clinical and genetic heterogeneity of amyotrophic lateral sclerosis. Clin Gene 2013; 83:408–416.
7. Wijesekera LC, Leigh PN. Amyotrophic lateral sclerosis. Orphanet J Rare Dis 2009; 4:3.
8. Shirakawa K, Suzuki H, Ito M, Kono S, Uchiyama T, Ohashi T, *et al*. Novel compound heterozygous ALS2 mutations cause juvenile amyotrophic lateral sclerosis in Japan. Neurology 2009; 73:2124–2126.

9. Andersen PM, Forsgren L, Binzer M, Nilsson P, Ala-Hurula V, Keränen ML, *et al*. Autosomal recessive adult-onset amyotrophic lateral sclerosis associated with homozygosity for Asp90Ala CuZn-superoxide dismutase mutation. A clinical and genealogical study of 36 patients. Brain 1996; 119:1153–1172.
10. Phukan J, Pender NP, Hardiman O. Cognitive impairment in amyotrophic lateral sclerosis. Lancet Neurol 2007; 6:994–1003.
11. Alavi A, Nafissi S, Rohani M, Zamani B, Sedighi B, Shamshiri H, *et al*. Genetic analysis and SOD1 mutation screening in Iranian amyotrophic lateral sclerosis patients. Neurobiol Aging 2013; 34:1516.e1–8.
12. Sapp PC, Rosen DR, Hosler B a, Esteban J, McKenna-Yasek D, O'Regan JP, *et al*. Identification of three novel mutations in the gene for Cu/Zn superoxide dismutase in patients with familial amyotrophic lateral sclerosis. Neuromusc Dis 1995; 5:353–357.
13. Andersen PM. Amyotrophic lateral sclerosis associated with mutations in the CuZn superoxide dismutase gene. Curr Neurol Neurosci Rep 2006; 6:37–46.
14. Wroe R, Wai-Ling Butler A, Andersen P, Powell JF, Al-Chalabi A. ALSoD: the amyotrophic lateral sclerosis online database. Amyotroph Lateral Scler 2008; 9:249–250.
15. Andersen PM, Al-Chalabi A. Clinical genetics of amyotrophic lateral sclerosis: what do we really know? Nat Rev Neurol 2011; 7:603–615.
16. Ince PG, Highley JR, Kirby J, Wharton SB, Takahashi H, Strong MJ, *et al*. Molecular pathology and genetic advances in amyotrophic lateral sclerosis: an emerging molecular pathway and the significance of glial pathology. Acta Neuropathol 2011; 122:657–671.
17. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, *et al*. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron 2011; 72:245–256.
18. Renton AE, Majounie E, Waite A, Simón-Sánchez J, Rollinson S, Gibbs JR, *et al*. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron 2011; 72:257–268.
19. Majounie E, Renton AE, Mok K, Dopper EG, Waite A, Rollinson S, *et al*. Frequency of the C9orf72 hexanucleotide repeat expansion in patients with amyotrophic lateral sclerosis and frontotemporal dementia: a cross-sectional study. Lancet Neurol 2012; 4422:1–8.
20. Ogaki K, Li Y, Atsuta N, Tomiyama H, Funayama M, Watanabe H, *et al*. Analysis of C9orf72 repeat expansion in 563 Japanese patients with amyotrophic lateral sclerosis. Neurobiol Aging 2012; 33:2527.e11–6.
21. Tsai C-P, Soong B-W, Tu P-H, Lin K-P, Fuh J-L, Tsai P-C, *et al*. A hexanucleotide repeat expansion in C9ORF72 causes familial and sporadic ALS in Taiwan. Neurobiol Aging 2012; 33:2232.e11–2232.e18.
22. Alavi A, Nafissi S, Rohani M, Shahidi G, Zamani B, Shamshiri H, *et al*. Repeat expansion in C9ORF72 is not a major cause of amyotrophic lateral sclerosis among Iranian patients. Neurobiol Aging 2014; 35:267.e1e267.e7
23. Al-Chalabi A, Lewis CM. Modelling the effects of penetrance and family size on rates of sporadic and familial disease. Hum Hered 2011; 71:281–288.
24. Rosen DR, Siddique T, Patterson D, Figlewicz D, Sapp P, Hentati A, *et al*. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 1993; 362:59–62.
25. Gellera C. Genetics of ALS in Italian families. Amyotroph Lateral Scler Other Motor Neuron Disord 2001; 2:S43–46.
26. Brooks BR, Miller RG, Swash M, Munsat TL. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotrophic Lateral Scler Other Motor Neuron Disord 2000; 1:293–299.
27. Costa J, Swash M, de Carvalho M. Awaji criteria for the diagnosis of amyotrophic lateral sclerosis:a systematic review. Arch Neurol 2012; 69:1410–1416.
28. Ticozzi N, Tiloca C, Morelli C, Colombrita C, Poletti B, Doretti A, *et al*. Genetics of familial Amyotrophic lateral sclerosis. Arch Ital Biol 2011; 149:65–82.
29. Sapp PC, Rosen DR, Hosler B a, Esteban J, McKenna-Yasek D, O'Regan JP, *et al*. Identification of three novel mutations in the gene for Cu/Zn superoxide dismutase in patients with familial amyotrophic lateral sclerosis. Neuromusc Disord 1995; 5:353–357.
30. Sabatelli M, Conte a, Zollino M. Clinical and genetic heterogeneity of amyotrophic lateral sclerosis. Clin Genet 2013; 83:408–416.