CASE REPORTS

An Italian kindred with FALS due to c.149T>C mutation in the SOD1 gene: case report of an affected family member

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We report the first Italian kindred with Familial Amyotrophic Lateral Sclerosis (FALS) due to c.149T>C mutation in the exon 5 of superoxide dismutase-1 (SOD1) gene. The proband was a 49-year-old woman who came to our observation because of an history of progressive limbs weakness and gait impairment. She belonged to a family of 24 affected members. The prevalent phenotype of the affected members was characterized by slowly progressive spinal impairment with proximal distribution of weakness, and bulbar involvement in advanced stages. We briefly reviewed the few previous reports about the same SOD1 mutation and discussed the hypothesis that structural instability of the mutant codon 149 protein may underlie some toxic effects significantly involved in FALS pathogenesis.

Key words: Familial ALS (FALS), superoxide dismutase 1 (SOD1), mutation

Introduction

Amyotrophic Lateral Sclerosis (ALS) is a progressive and fatal disease, characterized by degeneration of motor neurons. Around 5-10% of cases are considered to be familial (FALS) when the disease is present in both a proband and a first-degree or second-degree relative (1).

FALS is usually inherited in an autosomal dominant manner, though there are rarer cases of autosomal recessive and X-linked disease. FALS is genetically heterogeneous, including 15 mapped loci, of which the causative genes are identified for 11 (1). A number of genes has been recently identified, including TAR DNA binding protein/TDP-43 (TARDBP), fused in sarcoma/translocated in liposarcoma (FUS/TLS) and C9ORF72 (1, 2). Specifically, the large hexanucleotide repeat expansion in the first intron of the C9ORF72 gene is resulted the most common genetic cause of FALS. It was detected in more than one-third of FALS cases of European ancestry and in nearly one-half of Finnish FALS cases, unlike other gene mutations (2, 3).

Mutations in the copper/zinc superoxide dismutase-1 (SOD1, OMIM 147450) gene account for about 10% of FALS cases, though the frequency varies depending on the population sampled (1). To date more than 150 disease-causing mutations have been reported, spread throughout all five exons of the gene. They are mainly missense mutations but small deletions or insertions have also been described (www.alsod.org) (1). The phenotypes largely depend on the different mutations with significant intra and inter familial variability. However, a classical rapidly progressive ALS phenotype, clinically indistinguishable from sporadic disease, predominates (4).

Remarkably, more than 10 mutations in the SOD1 gene have been previously reported in FALS Italian patients (5), although no mutation has been observed in the exon 5.

Here we report a missense mutation c.149T>C in the exon 5 of the SOD1 gene, identified in an Italian patient with ALS belonging to a large family with FALS.

Case report

A 49-year-old woman (Fig. 1, individual IV-9) came to our observation with a five-years history of progressive limbs weakness and gait impairment.

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and IV-11 was extracted from leukocytes of whole blood samples. The remaining patients of the family did not give their consent or were not available for the analysis. The five coding exons of \textit{SOD1} gene and at least 30 bp of flanking intronic sequence, amplified by polymerase chain reaction (PCR), were sequenced using the Big-Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) and run on a capillary sequencer (ABI Prism 310 Genetic Analyzer, Applied Biosystems).

TARDBP, FUS/TLS and C9ORF72 genes were also screened to better characterize the genotypes of the three patients.

\textbf{Results}

DNA analysis of the proband and members IV-8 and IV-11 showed a heterozygous mutation c.149T>C in the \textit{SOD1} gene, causing a substitution of isoleucine to threonine (p.Ile149Thr).

Regarding TARDBP, FUS/TLS and C9ORF72, the three patients showed no pathologic mutations.

\textbf{Discussion}

We report the first Italian kindred of FALS due to exon 5 missense mutation c.149T>C in the \textit{SOD1} gene. Previously, the same mutation has been identified in a few Caucasian (7, 8) and Asian (9) families, and has been revealed able of inducing structural modifications of the relative charges of amino acids, significantly affecting the \textit{SOD1} enzymatic activity. In fact, about p.Ile149Thr mutation, it was found that the heterodimers composed by one normal and one mutant molecules appeared to be less efficient or
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Disease course, except for patient III-8 who had a more rapid disease progression. However, it should be taken into account that, in case of FALS, genotype-phenotype correlations have been established only for some SOD1 mutations and clear relations between mutations in critical residues and the age of onset or disease severity have not yet been identified (4).

Further analyses on this and other families with the same c.149T>C mutation of the SOD1 gene might help to explain its role in ALS pathogenesis and to evaluate the clinical and functional differences of these FALS phenotypes with those of sporadic ALS. Finally, in our as well in other similar cases, beyond the usual clinical management of the affected members, clinicians should be prepared to address also the needs of young subjects of the family who could consider to make genetic testing for this or other SOD1 mutations. Therefore, a genetic counseling should be planned to discuss the risks, benefits, and limitations of testing.

Table 1. Detailed patient characteristics.

| Patient | Sex | Age of onset (years) | Site of onset | Clinical presentation | Bulbar signs | Age of death (years) | Disease duration (years) |
|---------|-----|----------------------|---------------|-----------------------|--------------|---------------------|-------------------------|
| II - 2  | F   | NA                   | NA            | NA                    | NA           | 25                  | NA                      |
| II - 3  | M   | NA                   | NA            | NA                    | NA           | 25                  | NA                      |
| II - 5  | M   | NA                   | NA            | NA                    | NA           | 30                  | NA                      |
| II - 7  | M   | 36                   | LL            | NA                    | NA           | 36                  | 0.8*                    |
| II - 9  | M   | 48                   | Bulbar        | NA                    | +            | 48                  | 0.8                     |
| III - 2 | F   | NA                   | NA            | NA                    | NA           | 30                  | NA                      |
| III - 3 | F   | 40                   | LL            | LMN                   | -            | 49                  | 9                       |
| III - 6 | M   | 33                   | LL+UL         | UMN                   | -            | 40                  | 7                       |
| III - 8 | F   | 59                   | LL            | UMN                   | +            | 60                  | 1                       |
| III - 11| F   | NA                   | NA            | NA                    | NA           | 26                  | NA                      |
| III - 14| M   | NA                   | LL            | NA                    | +            | 36                  | NA                      |
| III - 17| M   | 48                   | LL            | NA                    | NA           | 50                  | 2*                      |
| III - 19| F   | 36                   | LL            | NA                    | NA           | 42                  | 5.5                     |
| III - 21| M   | 48                   | UL            | NA                    | +?           | 48                  | 0.1*                    |
| III - 26| F   | NA                   | NA            | NA                    | NA           | NA                  | NA                      |
| III - 28| F   | NA                   | NA            | NA                    | NA           | NA                  | NA                      |
| IV - 2  | F   | 49                   | LL            | UMN                   | +            | 50                  | 1                       |
| IV - 5  | M   | 39                   | LL            | NA                    | -            | 47                  | 8                       |
| IV - 8  | M   | 32                   | LL            | LMN                   | -            | living              | 9                       |
| IV - 9  | F   | 45                   | LL, proximal  | LMN+UMN               | -            | living              | 5                       |
| IV - 11 | M   | 38                   | LL, proximal  | LMN                   | -            | living              | 1                       |
| IV - 21 | F   | NA                   | NA            | NA                    | NA           | NA                  | NA                      |
| IV - 22 | F   | NA                   | NA            | NA                    | NA           | 40                  | NA                      |
| V - 2   | F   | 28                   | NA            | NA                    | NA           | 29                  | 1                       |

41.3±8.4 3.6±3.4

Abbreviations: LMN, lower motor neuron; UMN, upper motor neuron; LL, lower limb; UL, upper limb; and NA, data not available. Values in the last row represent mean ± standard deviation.

* Accidental death.

stable, causing a relevant destabilization of SOD1 dimer structure, and promoting the accumulation of toxic intracellular aggregates (7, 8). However, the exact mechanisms by which mutant SOD1 (mSOD1) causes motor neuronal cell death have yet to be established (1). Recently, evidence from transgenic models expressing mSOD1 has allowed to hypothesize a potential contribution of non-motor neuron cells, such as microglia, in triggering an alteration of the balance between neuroprotection and cytotoxicity in favor of the latter (10). In fact, misfolded proteins, such as mSOD1, seem to induce impairment of mitochondrial function and axoplasmic flow and release from motor neurons of abnormal signals able to activate microglia.

About genotype-phenotype correlations, in our case, the clinical presentation of the seven patients examined (III-3, III-6, III-8, IV-5, IV-8, IV-9, IV-11) was characterized by mean age of onset of 40.8 ± 9.1 years, spinal impairment with proximal distribution of weakness, bulbar involvement in advanced stages, and slowly progressive disease course, except for patient III-8 who had a more rapid disease progression. However, it should be taken into account that, in case of FALS, genotype-phenotype correlations have been established only for some SOD1 mutations and clear relations between mutations in critical residues and the age of onset or disease severity have not yet been identified (4).

Further analyses on this and other families with the same c.149T>C mutation of the SOD1 gene might help to explain its role in ALS pathogenesis and to evaluate the clinical and functional differences of these FALS phenotypes with those of sporadic ALS. Finally, in our as well in other similar cases, beyond the usual clinical management of the affected members, clinicians should be prepared to address also the needs of young subjects of the family who could consider to make genetic testing for this or other SOD1 mutations. Therefore, a genetic counseling should be planned to discuss the risks, benefits, and limitations of testing.
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