AMYOTROPHIC LATERAL SCLEROSIS AND SOD1 GENE: AN OVERVIEW

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KEY WORDS: Amyotrophic lateral sclerosis, SOD1, mutation, phenotype.

FUNCT NEUROL 2001;16 (SUPPL.): 171-180

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder affecting lower motor neurons in the spinal cord ventral horns and in the brainstem and upper motor neurons in the motor cortex. Clinically ALS is characterised by varying degrees of spasticity, weakness, wasting and atrophy of limb muscles. The muscles controlling speech, swallowing and respiration are also involved. Respiratory failure is normally the cause of death in the last stage of the disease (1). Current therapies have only a very limited effect on the clinical progression of the disease.

ALS history and epidemiology

Worldwide ALS prevalence is estimated to be about 4 to 6 per 100,000, with an annual incidence of 0.4 to 1.8 per 100,000 (2). Charcot first described ALS over 130 years ago as a sporadic neurodegenerative disease (3). Cases presenting with the inherited form of the disease have been recognised only since Kurland and Mulder’s comprehensive study in 1955 (4). Although the majority of ALS patients are sporadic (SALS), familial inheritance has been demonstrated in about 5-10% of cases (FALS), usually as an autosomal dominant trait. In a few cases a recessive transmission has been demonstrated (5-7).
**Hereditary forms of ALS**

Due to the severity of this neurological disorder, most FALS cases throughout the world belong to small families with few contemporary living affected members. This feature makes allele-genotyping studies difficult to perform. In a number of families showing a dominant transmission of the disease and a sufficiently extended pedigree for allele genotyping, linkage analysis studies identified a possible disease locus in a defined region on chromosome 21q, which contains the gene that encodes a cytosolic Cu/Zn-binding superoxide dismutase (SOD1) (8,9). This led to the identification of several SOD1 gene missense point mutations in the FALS families under study (10). Subsequent studies carried out on British (11) and American (12) families with inherited ALS have shown a SOD1 gene mutation in about 20% of cases.

The hereditary forms of ALS are now classified in two groups, respectively adult onset and childhood/juvenile onset forms. The first group comprises three dominant transmission forms:

- ALS-1 autosomal dominant transmission linked to sod1 gene on chromosome 21q22.1;
- ALS-3 comprises the remaining ALS cases with autosomal dominant transmission (about 80%), non-SOD1-linked;
- ALS-X dominant transmission linked to chromosome Xp11-q12.

The second group includes both dominant and recessive forms:

- ALS-2 autosomal recessive form with childhood onset and long survival, linked to chromosome 2q33;
- ALS-4 autosomal dominant form with juvenile onset and slow progression, linked to chromosome 9q34 (13);
- ALS-5 the most common of the ALS recessive forms, this is an autosomal recessive form with childhood onset, linked to chromosome 15q15.1-q21.1.

Linkage analysis carried out in the autosomal recessive ALS2 form led to the identification of the disease locus on chromosome 2q33. Recently a novel gene has apparently been identified within this region with 2 independent deletion mutations in 2 families, both leading to premature stop codon and loss of protein function.

In an American multicentre study, a set of families was considered in which subjects were found to develop both ALS and frontotemporal dementia (FTD), or either ALS or FTD alone. A genetic locus that is linked to ALS combined with FTD was identified on human chromosome 9q21-q22. Families with ALS alone did not show linkage to this locus (14).

**A LARGE ITALIAN KINDRED BEARING THE SOD1 L84F MUTATION**

In a small region of central Italy, we have identified an ALS cluster in which an underlying genetic defect (the SOD1 gene mutation L84F) was found to cause the spread of the pathological condition (15). In this area (400 square kilometres with a population of 166,151 inhabitants), we identified three living FALS and ten living SALS patients. The resulting prevalence was 7.8 per 100,000 inhabitants and was higher than the worldwide prevalence.

The three FALS cases, each leading to a different pedigree (kindreds A, B and C), appeared to be unrelated. However, after a detailed study of genealogical trees dating back to the 19th century, we discovered that kindred A and B were linked in generation II. Further genealogical studies failed to show any clear evidence of a relationship between family C and the other two families. Molecular investigations in all affected individuals led to the identification of the same L84F point mutation in the sod1 gene. Thus, the presence of the same gene point mutation in all three probands living in the same restricted area suggests that they have a common founder. If familial cases
are excluded, ALS prevalence in the area drops to 5.4/100,000, similar to the worldwide prevalence.

Together, the three kindreds have produced a total of 28 affected subjects spanning six generations. The disease is transmitted as an autosomal dominant trait. A penetrance of 90% was calculated by classic genetics and by sod1 sequencing in 10 subjects over 65 years of age at risk of inheritance (one subject carries the mutation, but is disease-free).

Clinical features

The clinical picture is unvarying and shows predominant lower motor neuron involvement and ascending progression of neurological deficits. Strength deficit begins in the lower limbs with weakness and muscular wasting, then spreads to the upper limbs and subsequently to the bulbar muscles. There is no gender prevalence. The duration of the illness varies considerably between 1.5 and 15 years with a mean of 4.8 ± 2.7 years (± SD). The age of onset ranges from 20 to 69 years with a mean of 42.8 ± 11.3 years. A significantly earlier onset of the disease (p<0.05) is evident when generation III (mean onset age 51.5 ± 8.4 years) is compared to generation V (mean onset age 33 ± 10 years).

Most sporadic ALS cases from the same area showed mixed lower and upper motor neuron damage and different sites of onset. One of them, a 61-year-old male of unknown father, presented clinical features resembling the FALS cases mentioned above: prevalent lower motor neuron involvement, onset at the lower limbs and an ascending progression of disease. He carried the same L84F SOD1 gene point mutation.

Generational anticipation

Epidemiological studies have identified an association between ALS and occupations that involve toxic exposure to agents such as lead and solvents (16-18). Moreover environmental toxicants may act upon a background of increased genetic susceptibility (17), and an excess of manganese and a low intake of magnesium have been associated with ALS (19). However, water analysis in the same area ruled out the presence of manganese, lead or other toxicants, and the mineral elements contained in the water were in the norm.

Shoe manufacturing, which involves the use of a wide range of adhesives and solvents, is widespread in the region where our L84F cases live. Chronic exposure to adhesives and solvents is common among affected and unaffected members of the last two generations. Nevertheless, none of them have shown signs of acute or chronic intoxication.

Although regional clustering of ALS does not seem to be due to toxic agents, the recent introduction of toxicants may be responsible for the generational anticipation characterising our families.

Pes cavus

Pes cavus was present in two affected FALS patients and in the healthy son of the A proband. In neurological disorders characterised by slowly progressive motor impairment of the legs, progressive muscular wasting may be accompanied by pes cavus (20,21). But usually ALS has a rapid course and pes cavus is not a prominent clinical feature. In our C proband foot deformity was absent before the onset of the disease. In this case pes cavus may be due to the slowly progressive muscular wasting of the legs (15 years). Conversely, the A proband was affected both by a rapidly progressive form of ALS and by a pre-existing foot deformity present also in the healthy son, who does not carry the L84F mutation. Thus, in this family, the pes cavus trait appears to segregate independently of the sod1 gene.
PHENOTYPE FEATURES OF OTHER FAMILIES CARRYING A MUTATION IN THE LEU84 CODON OF THE SOD1 GENE

The Leu84 codon

The alignment of SOD1 amino acid sequences of different species, from human beings to plants, shows a highly conserved Leucine in codon 84 (22). This codon is located in the C-terminal of the active site loop (IV) of the protein. The mutation is located in the sequence encoding for the active channel of the SOD1 enzyme. An L84 mutation can indirectly destabilise the Cu ligand His46 (one of the four His residues, which stabilise the enzyme centre: His46, His48, His63, His120), affecting enzyme structure and possibly resulting in a defective packing of the molecule (23, 24).

The L84F British family

Shaw describes a small family with two affected individuals over three generations (25). They carry the L84F mutation and show clinical features resembling those in the families reported by us (25). Age at onset was respectively 55 years in the father and 45 years in the daughter. Both developed progressive muscle weakness, beginning in the legs and spreading to the upper limbs. Disease duration was 5 years in the father. There is an apparent generational anticipation, but penetrance and variability in disease duration are difficult to assess because of scant patient number.

The L84V Japanese family

Abe et al. reported a Japanese family bearing a mutation in the same L84 codon of the SOD1 gene, with substitution of Leucine for Valine (22). They describe 5 affected members spread over three generations. Penetrance is complete. Mean age at onset is 53.8±15.3 years and there is an apparent generational antici-

CLINICAL FEATURES OF MAIN SOD1 MUTATIONS

The Ala 4 Val (A4V) mutation

Initially reported in North America, the A4V mutation is the most frequently occurring SOD1 gene point mutation in ALS. This mutation occurs in a stretch of the sod1 gene that is not highly conserved (24). Therefore, it might be a residue of minor importance to the normal enzyme function. Nevertheless, the A4V mutation is associated with a very poor survival rate and a rapidly progressive form of the disease. The duration of the disease from onset to death is also variable but generally less than 2 years (12,26). There is a substantial variation in the age at onset in the affected individuals belonging to families sharing this mutation. The age at onset was found to range from 55 to 68 years in a large family originating from central Sweden (27) and from 21 to 78 (mean 47.8±13.3) years in another report comprising 27 families from North America (12,28). Within a single family the disease can start in the upper limbs or in the lower limbs, or can initially affect the bulbar function (27). The progression to the other muscular districts does not follow an identical pattern. Dysarthria, dyspnoea, tongue atrophy with fasciculations, and facial muscle palsy can appear as initial signs or at the end of
the disease. All patients apparently show only lower motor neuron impairment with weakness and muscular atrophy, and with very weak or absent deep tendon reflexes. No pathological reflexes are present.

The Ile113Thr (I113T) mutation

The second most common mutation in the SOD1 gene is the Ile113Thr, found in British, North American, Australian, and New Zealand families. Attempts to trace a common founder of these families, scattered all over the world but possibly linked by the same molecular abnormality, have resulted in the identification of a possible common ancestor of Scottish origin who underwent the mutational event a few centuries ago (29,30).

The Ile113Thr mutation has also been reported in apparently sporadic ALS cases in Scotland (31). This finding could be explained by the low penetrance of this SOD1 gene mutation, by a high rate of occurrence of new mutation events, or by an uncompleted/non-detailed assessment of family history in these individuals.

Ile113Thr mutation is likely to disrupt dimeric interaction of the enzyme and therefore to produce a severe clinical form. A marked heterogeneity of the ALS clinical pattern in the Ile113Thr-mutated individuals has been reported, with a rapidly progressive course (6) and a mild form of ALS reported in the same pedigree of patients (9). The disease duration ranges from 3 to 20 years. In spite of the high variability of the clinical pattern, ALS patients with the Ile113Thr mutation seem to share a particularly advanced age at onset (58.9±12.6) in relation to other FALS-SOD1 related individuals (12).

The Gly93 codon

The Gly93 codon located in exon 4 seems to be particularly vulnerable, since all six of the possible single base changes in this position have been reported. The introduction of an amino acid with side chains in position 93 does not alter any restriction enzyme sites (32), but is critical for the stability of the conformation of the protein backbone (24). The clinical course of ALS patients bearing a SOD1 gene mutation with a change in the Gly93 position shows a high degree of variability depending on the type of amino acid substitution. Gly93Cys and Gly93Asp mutations are associated with a long survival rate and a similar mean disease duration: 10.1±6.2 years and 10.5±5.5 years respectively. Conversely, the Gly93Ala substitution is associated with shorter survival (2.2±1.5 years) (12). A marked variability in disease duration within and among FALS families with the Gly93Arg, Gly93Ser, and Gly93Val SOD1 mutations has been reported.

In the case of mutations occurring in codon Gly93, no clear correlation emerges between specific mutations and age at onset or disease duration, hence it is difficult to provide individual patients with a disease prognosis based on the detection of a specific mutation.

The Asp 90 Ala (D90A) mutation

The D90A mutation was first described in a Scandinavian population in which it was shown to determine ALS only in homozygous individuals, whereas the heterozygote carriers of this mutation showed only few or no clinical signs of the disease (33). In Belgium, the D90A heterozygote mutation was found in two families with ALS and in one sporadic case with a focal non-progressing ALS, suggesting that the D90A mutation is associated with an autosomal dominant pattern of inheritance (34).

Assuming that mutated SOD1 acquires a toxic function, a double dose of mutant protein would be expected to result in a more severe disease. However, homozygous patients from
recessive D90A families present a mild disease with most individuals surviving more than 10 years (33). Moreover, these recessive FALS patients are characterised by a homogeneous phenotype with predominantly lower motor neuron and lower limb involvement at onset. This finding is unusual since most SOD1 mutated heterozygous familial cases show phenotypic heterogeneity even within the same family. Disease phenotype for the heterozygous D90A families is not homogeneous.

Al-Chalabi identified 28 families with the D90A SOD1 allele, 8 of whom show dominant transmission. The two Scandinavian cases had bulbar onset, the UK case had mainly upper motor neuron involvement, while the three Belgian and two French cases showed a variable clinical presentation with intrafamilial heterogeneity and various disease progression patterns (35). The markedly different behaviour of the D90A mutation in FALS pedigrees of different origin has prompted the search for additional genetic factors which could act in association with the same SOD1 mutation determining a differential phenotypic expression. A haplotype study on both D90A autosomal recessive and autosomal dominant pedigrees was performed using six highly polymorphic microsatellite markers close to chromosome 21 sod1 gene location. A common founder was found only in the autosomal recessive FALS cases suggesting the presence of a co-segregating gene interacting with the phenotypic expression of the sod1 gene mutation (35).

Morita et al. reported a Japanese family bearing a mutation in the same D90 codon with substitution of Aspartate for Valine in the sod1 gene, the D90V mutation (36). The inheritance pattern is dominant. The clinical picture at onset is similar to the D90A heterozygous mutation and shows a lower limb involvement with an ascending trend. Disease duration is significantly shorter than D90A averaging between 2 and 3 years. Unlike the D90A mutation, which demonstrates normal SOD1 activity, the D90V mutation shows reduced SOD1 activity (5,34).

**SOD1 ACTIVITY AND AMOUNT IN THE L84F KINDRED**

SOD1 activity and amount in erythrocyte lysates were evaluated in 3 FALS subjects bearing the SOD1 L84F mutation, an asymptomatic case with the L84F mutation (L84F3) belonging to the same family, an apparently sporadic ALS patient (L84F4) with the SOD1 L84F mutation, SALS patients and controls. The mean total SOD activity (SOD1+ ECSOD) in plasma was not significantly different between SALS, FALS and control subjects. SOD1 activity (U/mg Hg) in RBC lysates paralleled results in plasma and was similar in SALS, controls and L84F patients. These findings suggest that SOD1 antioxidant activity is not crucial in triggering pathogenic mechanisms in FALS patients bearing the L84F mutation.

L84F1 and L84F3 display a high amount of mutated SOD1. They also presented an elevated SOD1 apparent catalytic efficiency (that is the ratio between activity and amount of SOD1 protein). L84F2 displays normal SOD1 apparent catalytic efficiency, therefore a low amount of mutated enzyme. He shows a slowly progressive form of the disease.

The set of analyses was performed two other times at intervals of 1 year. The results of the second and third sets of analyses in L84F1 and L84F3 suggest that a progressive loss of mutated SOD1 occurs during a worsening phase of the disease. The enzyme activity and apparent catalytic efficiency are reduced in comparison with the previous measurements and with control values. The amount of SOD1 is unchanged in the second analysis and only slightly lower in the third analysis. L84F2 has almost unchanged SOD1 activity and catalytic efficiency.
PLASMA AMINO ACID ANALYSIS IN THE L84F KINDRED

Amino acid content in plasma was evaluated in the above subjects and was performed two other times at intervals of 1 year. The plasma amino acid pattern did not differ between SALS and controls, whereas the L84F patients displayed elevated plasma levels of aspartate and glutamate.

L84F1 and L84F3 had the highest level of aspartate that dropped to normal at the second and third analyses. A rapidly evolving form of the disease characterises these patients. L84F2 had aspartate levels in the normal range. His disease progression during the three years considered was slow.

All 4 FALS patients had higher than normal values of plasma glutamate that remained elevated also at the second and third analysis. These results cannot be explained by dietary intake: fasting from the evening before should have minimised the effect of diet on the amino acid profile.

Our results demonstrate that plasma amino acid patterns are similar in SALS patients and controls. On the contrary, FALS patients tend to have higher levels of plasma glutamate and aspartate. Interestingly L84F5, who is somehow metabolically protected from neuronal damage induced by mutated SOD1, had remarkably low levels of aspartate, glutamate and branched chain amino acids. These findings suggest that a diet able to lower glutamate and branched chain amino acids could be beneficial in FALS patients bearing a SOD1 mutation.

MUTATED SOD1 PATHOGENIC MECHANISM IN THE L84F KINDRED

Over 70 mutations of the sod1 gene have been linked to familial ALS. ALS pathophysiology might include a complex interplay between different mechanisms. The onset and progression of the disease may reflect a heterogeneous genetic background that includes molecular abnormalities and predisposing factors. Environmental factors may also play a role (12,37). However, it is certainly true that single base changes, found in all of the five exons of the sod1 gene sequence, invariably give rise to the same well-defined disorder, which is the clinical manifestation of motor neuron degeneration. This means that every change in both functional and structural domains of the enzyme contribute to the same final effect, which is the involvement and death of motor nerve cells (25,27,38). Amino acid substitutions seem to occur both in gene sites which are highly conserved (like codon Leu84) and in gene sites which are mutational (like codon Gly93), but they do not appear to correlate with any distinctive clinical pattern.

The basic biochemical activity of SOD proteins is the conversion of potentially toxic superoxide radicals into hydrogen peroxide. Experimental evidence points to the gain of a toxic function for mutant SOD1 rather than inadequate oxygen radical scavenging as the primary pathogenetic mechanism in ALS (39). SOD1 mutations might affect the tightly folded structure of the enzyme and so decrease the ability of SOD1 to bind copper ions, which in turn, exert potent toxic effects against other proteins in the cell. In fact, the copper binding domain in SOD1 protein is highly conserved (40). Despite the experimental evidence for the role of abnormal copper binding in the aetiology of ALS, patients with a mutation in the copper binding site neighbouring codon 46 show clinical heterogeneity, including rapid disease progression in some pedigrees and slow progression of symptoms in others.

In the three FALS cases thoroughly examined in our study we found a correlation between the clinical progression of the disease, the amount of mutated SOD1 and excitotoxic amino acid metabolism. Massive reduction of
mutated SOD1 seems to accompany a fast worsening phase of the disease in the patients with the L84F mutation and may represent a defensive mechanism against the accumulation of toxic mutated protein. Alternatively, it may be due to precipitation and aggregation of the altered SOD1 protein itself.

Families carrying a SOD1 mutation that leads to ALS are a source of information valuable in furthering understanding of the role of mutated SOD1 in the pathogenesis of the disease. Furthermore, studies on subjects belonging to these families, bearing the SOD1 mutation and showing resistance to the development of ALS, could help to shed light on potential factors accounting for this protective effect.

REFERENCES


25. Shaw CE, Enayat ZE, Chioza BA et al. Mutations in all five exons of SOD-1 may cause ALS. Ann Neurol 1998;43:390-394


