European Journal of Human Genetics (1999) 7, 599–602

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SHORT REPORT

Mutational analysis of the Cu/Zn superoxide dismutase gene in 23 familial and 69 sporadic cases of amyotrophic lateral sclerosis in Belgium

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder caused by degeneration of motor neurons of the spinal cord and brain. The majority of ALS cases are sporadic (SALS). However, in 10–15% of ALS cases the disease is inherited as an autosomal dominant trait (familial ALS or FALS). We used a non-radioactive SSCP method, in combination with solid phase sequencing, to screen the entire *SOD1* (Cu/Zn superoxide dismutase) coding region and flanking intronic sequences for mutations. Twenty-three patients from 11 ALS families and 69 SALS patients of Belgian origin were studied. Three different mutations were identified (L38V, D90A and G93C) in seven families. Importantly, the D90A was only found in the heterozygous state. In addition two single base pair variants (IVS1 + 19G > A and AAC139 AAT) were identified in two SALS patients. These results suggest that the *SOD1* analysis is useful in FALS but less so in SALS cases. The SSCP analysis has proved fast and reliable for this purpose.

Keywords: amyotrophic lateral sclerosis; Cu/Zn superoxide dismutase; *SOD1*; neuromuscular disease; mutation analysis; variants

Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder primarily affecting motor neurons. The majority of ALS cases are sporadic (SALS). However, in 10–15% of ALS cases the disease is inherited as an autosomal dominant trait (FALS). In rare instances, a recessive inheritance has been documented. In a subset of the dominant FALS families the disease is caused by mutations in the gene encoding the anti-oxidant enzyme Cu/Zn superoxide dismutase (*SOD1*), located on the chromosome 21.[1,2] More than 57 different mutations in *SOD1* have been reported in familial cases affecting different functional domains of the enzyme.[3] In SALS cases, mutations have only rarely been found.[4] In the initial study of the involvement of the *SOD1* gene in FALS, two Belgian families were included with the L38V and G93C mutations respectively (families 11 and 57 in Rosen *et al*[2]). We here present a survey of nine additional Belgian ALS families. Also, in view of the importance for genetic

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Received 17 July 1998; revised 15 February 1999; accepted 19 February 1999

counselling, the *SOD1* gene of 69 patients with the sporadic form of ALS was screened.

Materials and Methods

Twenty-three affected individuals from 11 apparently unrelated families were available for mutation analysis. DNA was collected from 69 individuals with sporadic ALS. All patients included in this study were personally examined by one of our team (WR) and classified following the El Escorial criteria.[5] Fourteen patients had 'bulbar' onset, and in 55 patients onset was 'spinal'. The average age of onset was 61.5 ± 9.1 and 54.5 ± 13.9 years, respectively. The sex distribution was 33 females and 36 males. Seventy-five normal individuals were used as controls.

Genomic DNA was extracted from white blood cells of patients and controls using a salting-out method. Exons 2 and 4 from of the *SOD1* gene were amplified by PCR from genomic DNA as described.[2,6] Exons 1, 3 and 5 were analysed as described,[7] with minor modifications. Of each primer set, one primer was fluorescently labelled such that the fluorescent PCR product could be directly analysed by SSCP analysis.[6]

All PCR products presenting abnormal electrophoretic patterns and, for the familial cases, all exons and the exon–intron boundaries of *SOD1* were sequenced using a solid phase approach.[6] The fragments encompassing exons 1 and 3 were sequenced with the use of dITPs in the PCR amplification mix.[8]

Results

SOD1 *Mutations in Familial ALS*

SOD1 mutations were identified in seven of the 11 families (64%). Except for one family in which material from the parents was not available for analysis, the mutations in the familial cases were traced to previous generations. The L38V mutation was found in additional patients of the previously described family (results not shown).[2,9] The G93C mutation[2,9] was found in a total of four families (10 patients). The D90A mutation was found in two patients in two families. The clinical features of the latter patients have been described in detail.[6]

An additional SSCP variant was found in three of 23 (13%) of the patients (Figure 1A). It turned out to be a previously published polymorphism IVS3G +34A > C.[2] It was also present in 9.3% of the Belgian control group. Analysis of different members from the *SOD1* families allowed us to establish the phase between the *SOD1* mutant allele and the allele carrying the intronic polymorphism: in all cases the polymorphism was in a *trans* configuration.

SOD1 *Mutations in Sporadic ALS*

Of 69 SALS patients, nine showed an aberrant SSCP pattern. One SALS patient had the D90A mutation.[6] The other sequence variants represented silent mutations and polymorphisms. The presence of an intronic mutation (IVS1 +19A > G) (Figure 1B) was the only *SOD1* alteration found in a SALS patient, who had a bulbar onset at age 54. He died within 3.5 years of the onset. There is no information about the family history. In another SALS patient a C to T transition of the third base of codon 139 (exon 5) results in no amino acid change (Figure 1C). This patient had spinal onset at age 33 and died three years after onset. The family history is negative for neuromuscular disease. He is the only child of a couple aged 64 and 57 years. They are both healthy. These variants were not found in controls. Due to lack of material, further studies about the mRNA processing and/or stability were not possible. Six cases (8.7%) showed an aberrant SSCP pattern in exon 3, which corresponded to the intronic polymorphism IVS3 +34A > C already described (Figure 1A).

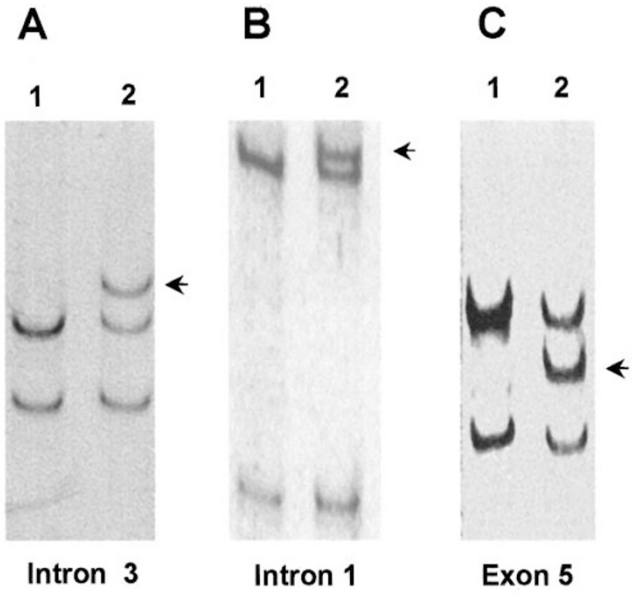

Figure 1 *A frequent polymorphism in intron 3 of the* SOD1 *gene and sequence variants in the* SOD1 *gene. Panel* **A***: IVS3 +34A > C was identified in FALS, SALS and control Belgian patients. Two variants were identified in SALS patients only: IVS1 +19A > G in intron 1 (panel* **B***) and AAC to AAT in codon 139 (exon 5, panel* **C***). 1: normal pattern; 2: aberrant pattern. Arrows indicate abnormal bands.*

Discussion

In this study we screened all five exons of *SOD1* in 11 apparently unrelated Belgian FALS families using a combination of SSCP and direct sequencing. The SSCP method has proved easy and reliable, as no other mutations have been found by direct sequencing, in the FALS cases. The L38V and G93C mutations are associated with classical FALS symptoms.

In two families (and in one apparently sporadic case) the D90A mutation was found in the heterozygous state. This mutation was first identified in Scandinavia in homozygotes with FALS suggesting a recessive effect.[10,11] It is associated with disease in the heterozygous state in our patients. However, the D90A mutation is found in families with non-classical features of the disease.[6] Direct sequencing of exons 1, 2, 3, 4 and 5 showed no additional mutations in the *SOD1* gene in these patients and this mutation was not found on 150 normal chromosomes. Population-based studies may help to understand the mechanism underlying the disease, other than the site of the mutation.[12]

A mutation in the *SOD1* gene in seven of 11 Belgian families (64%) is a high figure, compared with other previously published, larger studies, eg 13% in Canada or 20% in the UK,[13,14] but it is closer to the 50% in the Scottish series[15] and in agreement with the results of the original linkage analysis whereby in 55% of the FALS families linkage to markers on chromosome 21 was found.[16]

The most common mutation in the US, A4V, was not found in the Belgian population.[17] L38V and G93C have not (yet) been detected in other populations. A limited genealogical inquiry in the familial cases with the G93C mutation revealed that these families originated from a small region in Flanders, and probably have common ancestors. In contrast, in view of the wide occurrence of the enigmatic D90A mutation in Europe, a detailed haplotype analysis of the disease chromosomes is indicated to try to explain the regional variations in the phenotype of individuals carrying it.

We found three SALS cases with a sequence variant in the *SOD1* gene. The D90A mutation was found in one patient whose parents died of unrelated causes at the ages of 58 and 68.[6] The other two changes were only found in SALS: the IVS1 $+19A>G$ intronic variant and the silent mutation at codon 139. The latter mutation has also been reported recently in a Scandinavian ALS patient.[3] Although it should be considered a silent mutation, its involvement in the pathogenesis of the disease cannot be excluded. It should be noted that another silent mutation in codon 140 was the only change found in a familial case.[3] Until now a total of five synonymous codon mutations has been reported in *SOD1*,[3] but the recurrent presence of 'neutral' mutations in *SOD1* should be interpreted with caution. To ascertain the neutrality or non-pathogenicity of these apparent polymorphisms, their impact on mRNA processing and stability or even codon usage bias should be assessed.[18]

The frequency of the intronic polymorphism IVS3 $+34A>C$ in the Belgian control group (9.3%) seems to be of the same order of magnitude as in two other studies (4 and 11%).[15,17] However, three patients who harbour an exonic mutation and the polymorphism in *trans* configuration showed a remarkable rapidly progressive ALS. We suggest that it would be worthwhile to look at the association between this polymorphism and the disease phenotype in a larger sample of FALS with known mutations.

Acknowledgements

T Aguirre is supported by Fundación Gran Mariscal de Ayacucho, Venezuela. W Robberecht is a Clinical Investigator of the FWO Flanders.

References

1 Online Mendelian Inheritance in Man, OMIM (TM). Johns Hopkins University, Baltimore, MD. MIM Numbers: 105400, 602433, 205100, 602099, 147450. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/OMIM
2 Rosen DR, Siddique T, Patterson D *et al*: Mutations in the Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* 1933; **362**: 59–62.
3 Andersen PM, Nilsson P, Keränen M-L *et al*: Phenotypic heterogeneity in motor neuron disease patients with CuZn-superoxide dismutase mutations in Scandinavia. *Brain* 1997; **120**: 1723–1737.
4 Radunovic A, Leigh PN: Cu/Zn superoxide dismutase gene mutations in amyotrophic lateral sclerosis: correlation between genotype and clinical features. *J Neurol Neurosurg Psychiatry* 1996; **61**: 565–572.
5 Brooks BR: El Escorial World Federation of Neurology criteria for the diagnosis of amyotrophic lateral sclerosis. Subcommittee on Motor Neuron Diseases/Amyotrophic Lateral Sclerosis of the World Federation of Neurology Research Group on Neuromuscular Diseases and the El Escorial 'Clinical limits of amyotrophic lateral sclerosis' workshop contributors. *J Neurol Sci* 1994; **124** (Suppl): 96–107.

6 Robberecht W, Aguirre T, Van den Bosch L, Tilkin P, Cassiman J-J, Matthijs G: D90A heterozygosity in the *SOD1* gene is associated with familial and apparently sporadic amyotrophic lateral sclerosis. *Neurology* 1996; **47**: 1336–1339.
7 Yulug IG, Katsanis N, De Belleroche J, Collinge J, Fisher MC: An improved protocol for the analysis of *SOD1* gene mutations, and a new mutation in exon 4. *Hum Mol Genet* 1995; **4**: 1101–1104.
8 Dierick H, Stul M, De Kelver W, Marynen P, Cassiman J-J: Incorporation of dITP or 7-deaza dGTP during PCR improves sequencing of the product. *Nucleic Acids Res* 1993; **21**: 4427–4428.
9 Robberecht W, Sapp P, Viaene MK *et al*: Cu/Zn superoxide dismutase activity in familial and sporadic amyotrophic lateral sclerosis. *J Neurochem* 1994; **62**: 384–387.
10 Andersen PM, Nilsson P, Veli A-H *et al*: Amyotrophic lateral sclerosis associated with homozygosity for an Asp90Ala mutation in CuZn-superoxide dismutase. *Nat Genet* 1995; **10**: 61–66.
11 Själander A, Beckman G, Deng H-X, Iqbal Z, Tainer JA, Siddique T: The D90A mutation results in a polymorphism of Cu,Zn superoxide dismutase that is prevalent in northern Sweden and Finland. *Hum Mol Genet* 1995; **4**: 1105–1108.
12 Al-Chalabi A, Andersen PM, Chioza B *et al*: Recessive amyotrophic lateral sclerosis families with the D90A SOD1 mutation share a common founder: evidence for a linked protective factor. *Hum Mol Genet* 1998; **7**: 2045–2050.
13 Pramatarova A, Figlewicz DA, Krizus A *et al*: Identification of new mutations in the Cu/Zn superoxide dismutase gene of patients with familial amyotrophic lateral sclerosis. *Am J Hum Genet* 1995; **56**: 592–596.
14 Orrell RW, Habgood JJ, Gardiner I *et al*: Clinical and functional investigation of 10 missense mutations and a novel frameshift insertion of the gene for copper–zinc superoxide dismutase in UK families with amyotrophic lateral sclerosis. *Neurology* 1997; **48**: 746–751.
15 Jones CT, Swingler RJ, Simpson SA, Brock DJ: Superoxide dismutase mutations in an unselected cohort of Scottish amyotrophic lateral sclerosis patients. *J Med Genet* 1995; **32**: 290–292.
16 Siddique T, Figlewicz DA, Pericak-Vance MA *et al*: Linkage of a gene causing familial amyotrophic lateral sclerosis to chromosome 21 and evidence of genetic-locus heterogeneity. *N Engl J Med* 1991; **324**: 1381–1384.
17 Deng H-X, Hentati A, Tainer JA *et al*: Amyotrophic lateral sclerosis and structural defects in Cu,Zn superoxide dismutase. *Science* 1993; **261**: 1047–1051.
18 Richard I, Beckmann JS: How neutral are synonymous codon mutations? [Letter] *Nat Genet* 1995; **10**: 259.