ALS and other motor neuron disorders 2002 3, 69–74
 ISSN 1466-0822

A novel exon 3 mutation (D76V) in the SOD1 gene associated with slowly progressive ALS

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Received 4 February 2002
Revised 15 April 2002
Accepted 20 April 2002

INTRODUCTION: Details of the mutations in the Cu/Zn superoxide dismutase (SOD1) gene in patients with the familial form of amyotrophic lateral sclerosis are currently being gathered in order better to understand the genotype-phenotype relationship in this disorder. We report on a large family with 15 affected individuals spanning five generations.

RESULTS: A novel mutation in the exon 3 of the SOD1 gene, an A-to-T transversion at nucleotide position 696 in the heterozygous state leading to a D76V amino acid change, was identified in four family members. Affected individuals showed a homogeneous phenotype, characterized by initial symptoms in the lower limbs, clinical onset in the fifth decade of life, long survival and high penetrance.

DISCUSSION: Our results are discussed in relation to the previously reported exon 3 SOD1 mutations, paying particular attention to the phenotypic characteristics of ALS-SOD1 patients. (ALS 2002; 3: 69–74)

Keywords: familial amyotrophic lateral sclerosis – ALS-1 – SOD1 gene – D76V mutation – phenotype – MIM 105400

Introduction

The gene encoding Cu/Zn superoxide dismutase (SOD1) was the first reported gene linked with familial amyotrophic lateral sclerosis (FALS).[1–2] SOD1-associated FALS has been assigned to ALS-1 (MIM 105400). SOD1 gene mutations are nowadays thought to appear in approximately 20% of all FALS cases.

Other loci for some of the remaining 80% of FALS are being identified.[3–4] Some rare forms of autosomal recessive FALS with onset before age 25 are linked to chromosome 2q33-35 (ALS-2 [MIM 205100]) and to 15q15-q21 (ALS-5 [MIM 602099]).[5–7] Recently, it has been reported that alsin, a protein with three guanine-nucleotide exchange factor domains, is mutated in individuals with ALS-2 and in individuals with familial juvenile primary lateral sclerosis.[7] ALS-4 includes those forms of autosomal dominant juvenile FALS linked to chromosome 9q34.[8] ALS-3 includes forms of familiar autosomal dominant adult-onset FALS not linked to the SOD1 gene. A novel adult-onset FALS locus has recently been identified, mapped to the 18q21 region in a large European kindred not linked to SOD1 mutations.[9] Finally, other candidate genes for FALS include: 9q21-q22 for ALS, associated with fronto-temporal dementia [MIM 105550]);[10] 17q21-22 for disinhibition, dementia, parkinsonism and amyotrophy complex; a locus in Xp11-q12 for an X-linked dominant form with late onset mutations in the neurofilament heavy subunit (NF-H) gene (22q12.1-q13.1); and mutations in the glutamate transporter EAAT2 gene, for a small number of sporadic patients and isolated FALS cases.

At least 100 different mutations in SOD1 have been reported since 1993.[11–13] Most of these have been found to be associated with a dominant trait, although with sporadic/recessive cases. The majority of the genetic alterations described in the SOD1 gene are missense mutations but nonsense, deletional, insertional and non amino-acid altering mutations have been also described.[14] Approximately 30% of these point mutations are either an A-to-G or a G-to-A transition. Although mutations in all five exons of the SOD1 gene may cause ALS-1, exons 4 and 5 are considered hot spots for mutations. Conversely, mutations in exon 3 are relatively rare.

Variability in the disease's clinical features, including gender, age at symptom onset, site of onset, penetrance, and progression have been described in some ALS-1 families.[12,15,16] For this reason, clinical, genetic and neuropathological information is being gathered in the

ALSODatabase[13] in order to clarify the genotype-phenotype correlation in patients with SOD1 gene mutations.

Here we report on the identification of a novel missense mutation in SOD1 exon 3, an A-to-T transversion at nucleotide 696 leading to an aspartic acid to valine substitution at codon 76 (D76V) in a Spanish pedigree with 15 affected individuals spanning five generations. The slow progression of the disease observed in the family members who developed ALS suggests that the D76V mutation predicts a long survival.

Patients and methods

Patients

Figure 1 shows the pedigree of the family studied. The proband (IV:10) noticed muscle weakness in his right leg at age 46. Neurological examination revealed weakness and amyotrophy in the tibialis anterior muscle of the right leg, fasciculation in the four extremities and brisk tendon reflexes except in the right ankle. A left Babinski sign was also present. No sensory or cognitive impairment was detected. He complained of nocturnal cramps and paresthesias in the legs. The disease progressed over the next 6 years, affecting the left leg and the arms. Initial hand muscle weakness appeared at age 54. Dysarthria appeared at age 58.

Initial EMG findings at age 47 were consistent with a diffuse neurogenic disease, including fasciculations, denervation potential in the tibialis anterior and triceps suralis muscles in both legs, and in the interosseous muscle of the right hand. Sensory and motor conduction velocities were normal, without any sign of conduction block. Brain and spinal magnetic resonance imaging (MRI) showed no signs of motor neuron lesions. The disease progressed slowly over the next 15 years. At age 61, the patient was wheelchair-dependent. Dysphagia and ventilatory insufficiency appeared at age 69. The patient died at age 73.

Patient V:2, aged 49 when first examined, had noticed muscular weakness in the lower right extremity over the previous 18 months. He experienced problems in going up and down stairs. There was no history of back pain or speech disorders. On initial examination, he presented fasciculation in all four limbs, with generalized hyperreflexia. Babinski's sign and distal amyotrophy were present in the lower right limb. No bulbar, facial, or speech involvement were present. The patient needed a stick for walking. EMG revealed signs of denervation in all four extremities. Sensory and motor nerve conduction were normal. No conduction blocks were present. A transcranial magnetic simulation (TMS) showed an abnormally prolonged central motor conduction time. Brain and cervical MRI were normal.

Individual V:4, a healthy 45-year-old man, was examined by one of our neurological team (JG). This family member did not complain of any weakness, cramps or fasciculation. Neurological examination disclosed no signs of motor neuron disease. EMG showed only occasional fasciculation in the right triceps suralis. The remainder of the neurophysiological examination, including TMS, showed no abnormalities.

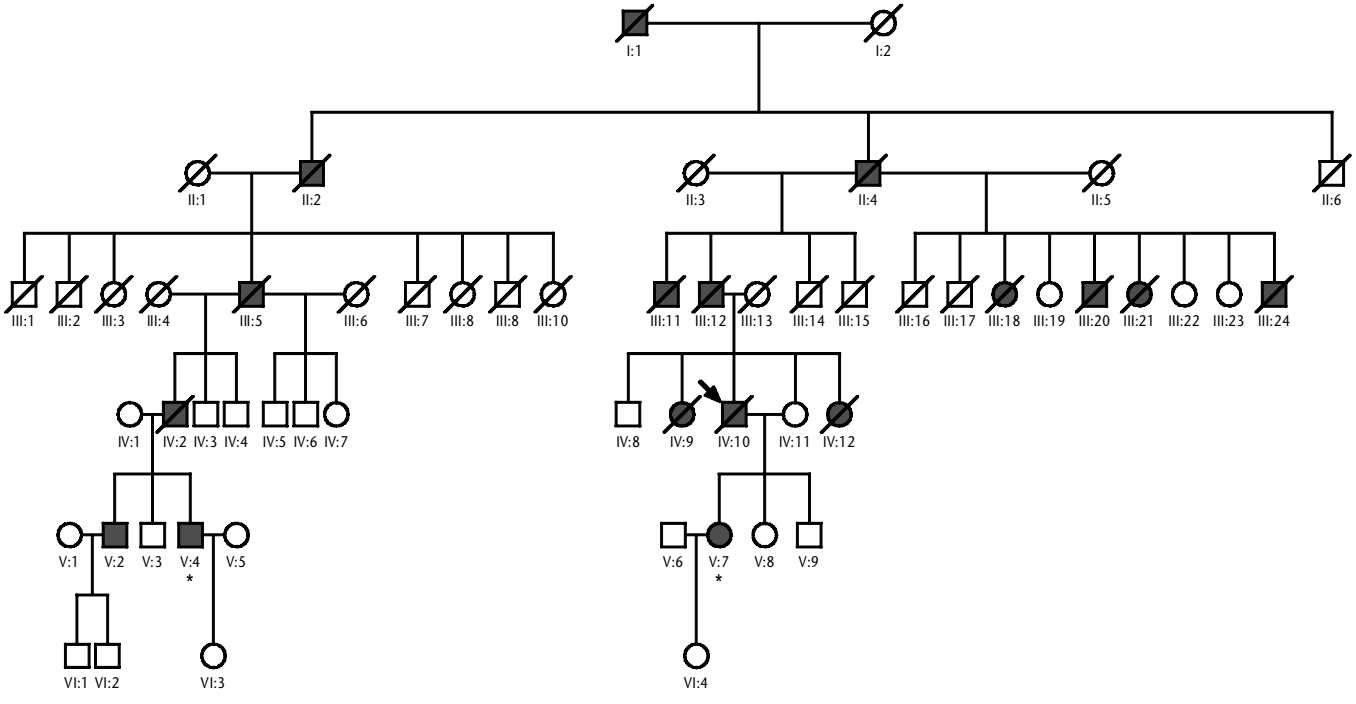

Figure 1

Pedigree of the family studied. Over 15 members developed ALS. The proband is indicated by an arrow (IV:10). Affected members are represented by solid squares or circles. Asymptomatic individuals harbouring the mutation are indicated by an asterisk (V:4 and V:7).

Case no.	Gender	Site of onset	Age at onset (years)	Age at death (years)
IV:10	M	RLL	46	73
IV:2	M	LLL	45	65
IV:9	F	LL	43	51
V:2	M	RLL	47	Alive
II:4	F	LL	49	69

Table 1
Clinical characteristics of pedigree members with known clinical course. LLL: left lower limb; LL: lower limbs; RLL: right lower limb.

The family history disclosed over 15 individuals over five generations affected with ALS, with a dominant inheritance pattern (Figure 1). Although this family was spread throughout Spain, one of the more distant affected ancestors originally came from northern Castile, in central Spain. Clinical details, including age at onset of first symptom, initial topography and progression were available from five affected family members, and age at death was available for four (Table 1).

Complete information regarding the clinical course of the disease in the remaining affected family members was unavailable; of the dead affected, the majority were male (10 M/4F).

Genetic investigations

Blood samples were taken from the five available members (IV:10, V:2, V:4, V:7 and V:9) of the pedigree after informed consent and with the permission of our hospital's Ethical Committee. The control population group consisted of 150 age-matched subjects recruited among spouses and non-related carers of ALS patients. The sporadic ALS group comprised 110 individuals aged between 36 and 84 years. All individuals – the control population and sporadic ALS groups – were caucasian, originating from different regions of Spain. Genomic DNA was extracted from the controls' and affected individuals' blood following standard procedures. Polymerase chain reaction (PCR) fragments encompassing the entire coding region of the SOD1 gene (exons 1 to 5) were obtained using five sets of 20-mer primers. The fragments were subsequently sequenced on an ABI Prism 310 genetic analyzer (Perkin Elmer, CA, USA). To confirm the presence of the mutation, we performed restriction-fragment-length polymorphism (RFLP) analysis using a 24-mer mismatched forward primer (5'-CCAgAAAACACggTgggCCAAA*C*g-3') and a 21-mer reverse primer (5'-gCAAAggTgggggAAACACgg-3'). In the presence of the mutation, the mismatched primer creates a novel restriction site for the *Psp*1406 I enzyme (Takara Biomed, Shiga, Japan). PCR products were digested overnight with the restriction enzyme at 37°C and resolved in a 12% polyacrylamide gel. The presence of the heterozygous mutation was confirmed by the appearance of two bands of 143 and 123 bp on the gel.

Biochemical studies

Erythrocyte SOD1 activity was measured in individuals V:2 and V:4 using the RANSOD SD125 kit (Randox Laboratories Ltd. Crumlin, UK)[17] and expressed as international units (IU) per gram of protein. The protein concentration was measured using the Bradford method.[18]

Results

Sequence analysis of the SOD1 gene revealed an A-to-T transversion at nucleotide position 696 in the heterozygous state in four family members. The mutation was absent in 110 sporadic ALS cases, 150 normal individuals and in 20 probands belonging to dominant FALS families with at least three affected generations. Due to the nucleotide change, a valine residue replaces the wild-type aspartic acid residue at position 76 (D76V). The presence of the mutation was assessed by sequencing the reverse strand of exon 3 (Figure 2) and confirmed by RFLP analysis. As shown in Figure 3, the proband (IV:10), another 49-year-old affected male (V:2) and two asymptomatic family members – a 45-year-old man (V:4) and a 30-year-old woman (V:7) – were D76V mutation carriers.

SOD1 activity in patient V:2 (396.8 IU/g protein) and in his asymptomatic brother (V:4) harbouring the mutation was lower than in the control group (54.9% and 68.1% respectively).

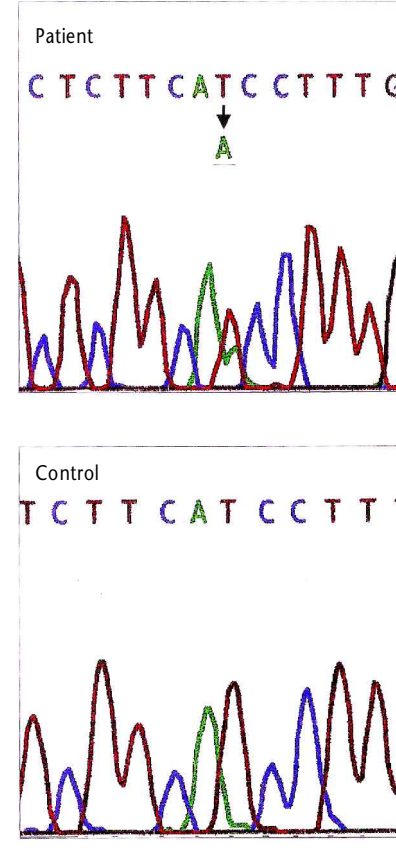

Figure 2
Electropherogram of the SOD1 gene in exon 3, showing the DNA sequence in a control subject and in a patient. The T-to-A transversion (reverse sequence) at nucleotide 696 is indicated by an arrow.

Original Research

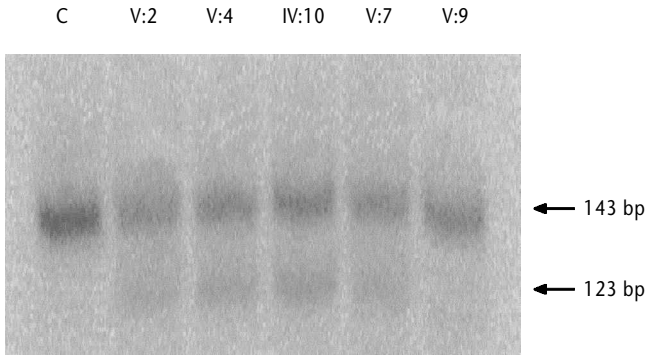

Figure 3
Restriction fragment length polymorphism analysis of the 143-bp fragment amplified by PCR. A restriction site for Psp1406 I is created in patients with the mutation. As the mutation was heterozygous, three DNA fragments of 143,123 and 20 bp were obtained after enzymatic digestion. Only the two major bands were resolved in a 8% polyacrylamide gel. (C 5 = control).

Discussion

We report on the identification of a novel SOD1 gene missense mutation in a large Spanish kindred showing autosomal dominant FALS. The pathogenicity of the D76V mutation is suggested by the following findings.

First, it causes a hydrophobic-to-neutral amino acid change in a sensitive domain of the protein. Residue D76 is located within the Zn-subloop, in loop IV of the SOD1 protein. The Zn-subloop (residues 49-84) and loop VII (residues 121-144) constitute the enzyme's active site channel. As D76 is located in a turn of a solvent-exposed loop, the introduction of a hydrophobic valine residue would eventually lead to an improper folding of loop IV. D76 forms an electrostatic interaction with the K128 amino group at loop VII. This union between the two loops could be suppressed by the effect of the D76V mutation, which introduces a hydrophobic environment at the mouth of the electrostatic channel. Superoxide anion diffusion through the active site channel would thus be greatly impaired in D76V-SOD1 mutants. In this regard, a decreased SOD1 enzyme activity was found in both the proband and his asymptomatic brother. Although one of the suggested pathogenic mechanisms to explain the disease is a gain in the toxic function of mutated SOD1,[19] many of the mutations in kindred with FALS are associated with a decreased activity in the erythrocytes.

Second, D76 is a well-preserved residue in different organisms (Table 2). When 106 different superoxide dismutase protein sequences were analysed by the MaxHom alignment tool[20] (PredictProtein server; http://maple.bioc.columbia.edu/predictprotein), this residue was included within the 70% consensus sequence. The Zn subdomain of the IV loop presents several well-preserved residues, including nine almost invariant amino acids (H50, G53, G61, H63, P66, H71, H80, G82 and D83) that appear in the 90% consensus sequence.

Over 60% of all SOD1 mutations have been found at exons 4 and 5. However, only five exon 3 mutations (S59S, N65S, L67R, G72S, and D76Y) have been reported.[13,21–26] Various explanations have been suggested for the low frequency of mutations in this exon 3. Among them are reduced penetrance, association with a benign clinical course, *in-utero* high toxicity of these mutations leading to early miscarriage, or even technical difficulties (PCR-primer set and/or single stranded confirmation polymorphism (SSCP) running conditions) in detecting exon 3 mutation.[26–27] Four of these five exon 3 mutations are missense mutations.

For some SOD1 mutations there is a good genotype-phenotype correlation. For others the same mutation can show a different clinical course and a variable disease penetrance. Furthermore, an intrafamilial heterogeneity is a common feature in certain SOD1 mutations.[13,28] In previously identified mutations in exon 3, age at onset, symptoms presented and survival were heterogeneous. No clear phenotype-genotype correlation was observed. The phenotype was rather that of sporadic ALS. In this regard, in most of the families reported only one member was found to be affected, and was considered as an apparently sporadic case.

In 1997, Orrell Marklund and de Belleroche reported the first mutation in exon 3 (Gly72Ser) as a result of an A-to-G substitution.[22] The family described showed two affected individuals belonging to the same generation with initial symptoms in the lower limbs, predominantly lower motor neuron signs, and onset at the end of the fifth

	50									60										70										80									
Patient	V	H	E	F	G	D	N	T	A	G	C	A	G	P	H	F	N	P	L	S	R	K	H	G	G	P	K	**V**	E	E	R	H	V	G	D	L
Human	V	H	E	F	G	D	N	T	A	G	C	A	G	P	H	F	N	P	L	S	R	K	H	G	G	P	K	**D**	E	E	R	H	V	G	D	L
Bovine	V	H	Q	F	G	D	N	T	Q	G	C	A	G	P	H	F	N	P	L	S	K	K	H	G	G	P	K	**D**	E	E	R	H	V	G	D	L
Mouse	V	H	Q	Y	G	D	N	T	Q	G	C	A	G	P	H	F	N	P	H	S	K	K	H	G	G	P	A	**D**	E	E	R	H	V	G	D	L
Chicken	V	H	E	F	G	D	N	T	N	G	C	A	G	A	H	F	N	P	E	G	K	Q	H	G	G	P	K	**D**	A	D	R	H	V	G	D	L
Xenopus l.	I	H	V	F	G	D	N	T	N	G	C	A	G	P	H	F	N	P	E	N	K	N	H	G	A	P	G	**D**	T	D	R	H	V	G	D	L
Drosophila m.	V	H	E	F	G	D	N	T	N	G	C	S	G	P	H	F	N	P	Y	G	K	E	H	G	A	P	V	**D**	E	N	R	H	L	G	D	L
Saccharomyces c.	I	H	E	F	G	D	A	T	N	G	C	A	G	P	H	F	N	P	F	K	K	T	H	G	A	P	T	**D**	E	V	R	H	V	G	D	M

Table 2
Sequence comparison of the loop IV (residues 49-84) of Cu/Zn superoxide dismutase in different species.

decade. The duration of the disease was 4 years in the proband and 2–3 years in his sister. No details of other possibly affected members, especially from previous generations, were available. As in our family, erythrocyte SOD1 activity was also found to be decreased.

A year later, Shaw and colleagues reported a second family harbouring the same exon 3 mutation but with a different phenotype, in an apparently sporadic ALS kindred in which the affected case was a 29-year-old man who had received human pituitary growth hormone treatment as a child.[23] He presented pain and weakness in his left thigh, and died 15 months after the appearance of the initial symptom.

Andersen and co-workers reported two motor neuron disease (MND) cases in a Danish family carrying a heterozygous G-to-T transversion at nt 695.[24] The proband in this family had initial symptoms in the right foot at the age of 49. Fifteen years after clinical onset, the patient was severely tetraparetic with dysphonia and dyspnoea, and needed to use an electric wheelchair controlled by his left hand. His mother did not develop MND, but his maternal grandfather developed a pseudobulbar syndrome with dysarthria, dysphonia and affective lability at age 67. As the disease progressed, he had difficulties in walking, and died 15 months after the appearance of the initial symptom.

No details regarding the number of affected members and their clinical profile in the kindred carrying the third missense mutation in exon 3 described (Leu67Arg) have been reported.[25]

Finally, the N65S was found in a caucasian male patient from Zimbabwe, with initial symptoms in the right leg at 34 years of age (he is still alive 97 months after the onset of symptoms) and in a USA FALS pedigree with reduced penetrance. The amino acid substitution is the result of an A-to-G nucleotide change.[13,26]

The remaining exon 3 SOD1 mutation reported (S59S) is a neutral polymorphism (AGT→ AGC) that was found in an affected individual of an ALS1 family harbouring the G37R mutation (exon 2).[21] Although the role of the S59S mutation has yet to be demonstrated, most SOD1 gene mutation lists include it in the polymorphisms or unverified mutations section.[11–13]

By contrast, in the family described here, the affected subjects for whom information is available showed a homogeneous phenotype characterized by onset of symptoms in the fifth decade of life, initial symptoms affecting the lower extremities, relatively late bulbar muscle impairment, and slow progression. The mean age of onset of symptoms was 46.6 years and mean survival time was 17.25 years. Although individual V:4 is at present asymptomatic, he shows a decreased SOD1 activity. Due to the fact that at the age of 45 he is now getting close to the critical age for clinical onset, he is currently being followed up by our ALS unit. The age of the other asymptomatic carrier (a 30-year-old woman) is much lower then the average age at onset. In this case it was not possible to measure SOD1 activity. The present data for D76V is consistent with dominant inheritance, with high if not complete disease penetrance. This contrasts with the previously published D76Y, which showed a variable phenotype and incomplete penetrance.

A male predominance was initially interpreted in our family: ten of its 14 members known to have died affected with ALS were men. Although in certain families the literature shows a predominance of either men or women, FALS is reported to occur in males and females with approximately the same frequency. It has been suggested that oestrogen hormones have a neuroprotective effect in reducing lipid peroxidation in the central nervous system[29] and an anti-apoptotic function,[30] which would explain a potential gender bias. Other genetic or epigenetic factors intrinsic to the mutation itself may influence the expressivity of the mutation and should not be ruled out. However, a comparative analysis of the number of patients grouped by gender showed that nine of the 23 males (39%) belonging to the second, third and fourth generations had died as a result of the disease, while only four out of 11 women of the same generations (36%) had died from it.

The identification of the responsible mutation would have special significance for individuals at risk. Prenatal diagnosis would thus be possible for family members when a SOD1 mutation, especially one of those mutations predicting a homogeneous phenotype and complete penetrance, is identified in their pedigree. Furthermore, in those families carrying mutations associated with high penetrance for which a long survival time can be predicted, it would be possible to perform trials of preventive treatments for asymptomatic carriers and to obtain better information regarding the disease's pre-clinical phase[31–33]

Conclusion

D76V is the first SOD1 novel mutation reported in FALS in Spain. Routine screening of ALS patients for SOD1 mutations in the clinical laboratory will help characterize the impact of this and other SOD1 mutations on the ALS phenotype and their relative frequency among different ethnic groups.

Acknowledgements

We are grateful to Dr Peter M Andersen of the Department of Neurology, Umeå University Hospital, Sweden, for his review of the manuscript and suggestions. This study has been supported by a grant from the Spanish *Fondo de Investigaciones Sanitarias* (FIS00/0797). The first author is the recipient of a fellowship from the Vall d' Hebron Foundation. The authors are indebted to the members of this family for their collaboration.

References

1. Rosen DR, Siddique T, Patterson D et al. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 1993; 362: 59–62.
2. Deng HX, Hentati A, Tainer JA et al. Amyotrophic lateral sclerosis and structural defects in Cu,Zn superoxide dismutase. Science 1993; 261: 1047–1051.
3. Shaw PJ. Genetic inroads in familial ALS. Nature Genet 2001; 29: 103–104.
4. Hand CK, Rouleau GA. Familial amyotrophic lateral sclerosis. Muscle Nerve 2002; 25: 135–159.
5. Hentati A, Ouahchi K, Pericak-Vance MA et al. Linkage of a commoner form of recessive amyotrophic lateral sclerosis to chromosome 15q15-q22 markers. Neurogenetics 1998; 2: 55–60.
6. Yang Y, Hentati A, Deng HX et al. The gene encoding alsin, a protein with three guanine-nucleotide exchange factor domains, is mutated in a form of recessive amyotrophic lateral sclerosis. Nature Genet 2001; 29: 160–165.
7. Hadano S, Hand CK, Osuga H et al. A gene encoding a putative GTPase regulator is mutated in familial amyotrophic lateral sclerosis 2. Nature Genet 2001; 29: 166–173.
8. Blair IP, Bennett CL, Abel A et al. A gene for autosomal dominant juvenile amyotrophic lateral sclerosis (ALS4) localizes to a 500-kb interval on chromosome 9q34. Neurogenetics 2000; 3: 1–6.
9. Hand CK, Khoris J, Salachas F et al. A novel locus for familial amyotrophic lateral sclerosis, on chromosome 18q. Am J Hum Genet 2002; 70: 251–256.
10. Hosler BA, Siddique T, Sapp PC et al. Linkage of familial amyotrophic lateral sclerosis with frontotemporal dementia to chromosome 9q21-q22. JAMA 2000; 284: 1664–1669.
11. Orrell RW. Amyotrophic lateral sclerosis: copper/zinc superoxide dismutase (SOD1) gene mutations. Neuromuscul Disord 2000; 10: 63–68.
12. Gaudette M, Hirano M, Siddique T. Current status of SOD1 mutations in familial amyotrophic lateral sclerosis. ALS 2000; 1: 83–89.
13. http://www.alsod.org
14. Watanabe Y, Kato S, Adachi Y, Nakashima K. Frameshift, nonsense and non amino acid altering mutations in SOD1 in familial ALS: report of a Japanese pedigree and literature review. ALS 2000; 1: 251–258.
15. Radunović A, Leigh PN on behalf of the European Familial ALS Group. Cu/Zn superoxide dismutase gene mutations in amyotrophic lateral sclerosis: correlation between genotype and clinical features. J Neurol Neurosurg Psychiatry 1996; 61: 565–572.
16. Cudkowicz ME, McKenna-Yasek D, Sapp PE et al. Epidemiology of mutations in superoxide dismutase in amyotrophic lateral sclerosis. Ann Neurol 1997; 41: 210–221.
17. Suttle NF, McMurray CH. Use of erythrocyte copper:zinc superoxide dismutase activity and hair or fleece copper concentrations in the diagnosis of hypocuprosis in ruminants. Res Vet Sci 1983; 35: 47–52.
18. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye-binding. Anal Biochem 1976; 72: 248–254.
19. Wiedau-Pazos M, Goto JJ, Rabizadeh S et al. Altered reactivity of superoxide dismutase in familial amyotrophic lateral sclerosis. Science 1996; 271: 515–518.
20. Sander C, Schneider R. Database of homology-derived structures and the structural meaning of sequence alignment. Proteins 1991; 9: 56–68.
21. Hosler BA, Nicholson GA, Sapp PC et al. Three novel mutations and two variants in the gene for Cu/Zn superoxide dismutase in familial amyotrophic lateral sclerosis. Neuromuscul Disord 1996; 6: 361–366.
22. Orrell RW, Marklund SL, deBelleroche JS. Familial ALS is associated with mutations in all exons of SOD1: a novel mutation in exon 3 (Gly72Ser). J Neurol Sci 1997; 153: 46-49.
23. Shaw CE, Enayat ZE, Chioza BA et al. Mutations in all five exons of SOD1 may cause ALS. Ann Neurol 1998; 43: 390–394.
24. Andersen PM, Nilsson P, Keränen ML et al. Phenotypic heterogeneity in motor neurone disease patients with CuZn-superoxide dismutase mutations in Scandinavia. Brain 1997; 120: 1723–1737.
25. Boukaftane Y, Khoris J, Moulard B et al. Identification of six novel SOD1 gene mutations in familial amyotrophic lateral sclerosis. Can J Neurol Sci 1998; 25: 192–196.
26. Andersen PM. Genetics of sporadic ALS. ALS 2001; 2 (Suppl 1), S37–S41.
27. Siddique T, Nijhawan D, Hentati A. Molecular genetic basis of familial ALS. Neurology 1996; 47 (Suppl 2): S27–S35.
28. Orrell RW, King AW, Hilton DA, Campbell MJ, Lane RJM, de Belleroche JS. Familial amyotrophic lateral sclerosis with a point mutation of SOD-1: intrafamilial heterogeneity of disease duration associated with neurofibrillary tangles. J Neurol Neurosurg Psychiatry 1995; 59: 266–270.
29. Vedder H, Anthes N, Stumm G, Wurz C, Behl C, Krieg JC. Estrogen hormones reduce lipid peroxidation in cells and tissues of the central nervous system. J Neurochem 1999; 72: 2531–2538.
30. Sawada H, Shimohama S. Neuroprotective effects of estradiol in mesencephalic dopaminergic neurons. Neurosci Biobehav Rev 2000; 24: 143–147
31. Orrell RW, Figlewicz DA. Clinical implications of the genetics of ALS and other motor neuron disease. Neurology 2001; 57: 9–17.
32. Rowland LP. Six important themes in amyotrophic lateral sclerosis (ALS) research, 1999. J Neurol Sci 2000; 180: 2–6.
33. Rowland LP, Shneider NA. Amyotrophic lateral sclerosis. N Engl J Med 2001; 344: 1688–1700.