Superoxide dismutase mutations in an unselected cohort of Scottish amyotrophic lateral sclerosis patients

Cheryl T Jones, Robert J Swingler, Sheila A Simpson, David J H Brock

Abstract

Mutations in the Cu/Zn superoxide dismutase (SOD1) gene are responsible for some cases of familial amyotrophic lateral sclerosis (ALS). We have shown that SOD1 mutations can also occur in apparently sporadic ALS. To establish how often this happens we have undertaken a study of the prevalence of SOD1 mutations in an unselected cohort of Scottish ALS patients, with both sporadic (n=57) and familial (n=10) disease. Single strand conformation polymorphism analysis was used to scan for new mutations, and selective restriction enzyme digestion to screen for 11 of the 20 SOD1 mutations published to date. We detected mutations in five (50%) of the familial ALS patients and also in four (7%) of the sporadic patients. One mutation, ile113thr, seems to be particularly prevalent in the Scottish population since it was detected in a total of 6/67 (9%) unrelated cases.

(*J Med Genet* 1995;32:290-292)

Human Genetics Unit,
University of
Edinburgh, Western
General Hospital,
Edinburgh EH4 2XU,
UK

C T Jones
D J H Brock

Department of
Clinical Neuroscience,
University of
Edinburgh Western
General Hospital,
Edinburgh EH4 2XU,
UK

R J Swingler

Department of
Medical Genetics,
Clinical Genetics
Service, Medical
School, Aberdeen
Royal Hospitals NHS
Trust, Foresterhill,
Aberdeen AB9 2ZD,
UK

S A Simpson

Correspondence to:
Professor Brock.

Received 20 August 1994
Revised version
accepted for publication
19 December 1994

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal paralytic disorder characterised by degeneration of motor neurones in the brain and spinal cord. The majority of cases are of a sporadic nature, while around 5 to 10% of cases are familial with the disorder inherited as an autosomal dominant trait with age dependent penetrance.¹ Sporadic and familial forms are clinically indistinguishable with death usually resulting between two and five years after onset of the illness.

Linkage to chromosome 21q22.1 was observed for a subset of ALS families in the USA, showing a degree of genetic heterogeneity among the familial forms.² In 1993 a collaborative effort between several groups in the USA showed that mutations in the superoxide dismutase 1 gene (SOD1) segregated with the disease in several ALS families.³ This enzyme serves to scavenge the damaging superoxide free radicals generated by normal cellular processes, these being among several free radical species thought to be implicated in many neurodegenerative disorders.⁴

The SOD1 gene comprises five small exons⁵ making it ideal for mutation screening. Since the initial report,³ a total of 20 missense mutations has been published.⁶⁻¹⁰ SOD1 assays performed on red blood cells from heterozygous patients showed a reduction below 50% in enzyme activity.⁶ Although most loss of function mutations have a recessive rather than a dominant effect, SOD1 is a homodimeric enzyme. Thus mutations may exert a dominant negative effect,¹¹ that is, the product from the mutant allele disrupts the activity of the normal product in the dimeric enzyme. Indeed, several of the mutations mapped onto the human crystallographic structure of SOD1 have been shown to be directly involved in dimer contact.⁶

The fact that we detected three germline mutations in patients whose ALS was not thought to be inherited⁷ has important implications for the management of the disorder. We therefore decided to carry out a survey of SOD1 mutations in a population based cohort of ALS patients. To our knowledge this has not been done before.

Methods

AMPLIFICATION OF PATIENT GENOMIC DNA

DNA was extracted from peripheral blood leucocytes or transformed lymphoblast cell culture pellets using standard techniques. Approximately 200 ng of DNA was PCR amplified for 30 cycles using annealing conditions specific for each primer pair.

Primers and PCR conditions for exon 1,⁶ exon 2 (set "b"),³ and exon 4 (set "a")³ were as described. The primers used for exons 3 and 5 were:

exon 3:

3A-5' CTTCTTCTTATAAATAGGCTG 3'
3B-5' AGGCACATATTTACAAGTAGT 3'

exon 5:

5A-
5'GTGATTACTTGACAGCCCAAAG3'
5B-
5'CAGGATACATTTCTACAGCTAG3'.

A restriction site generating primer, 5' TCACTCTCAGGAGACCATTGCATCC 3' (RG113), was used in conjunction with the

Table 1 Age and cause of death of parents of patients with apparently sporadic ALS and SOD1 mutations

Ile113thr	
CL	Mother: 55 years, hypertension Father: 68 years, prostatic carcinoma
VM	Mother: 71 years, pulmonary embolus, heart failure Father: 62 years, pulmonary embolus, deep vein thrombosis
AN	Mother: 55 years, hernia peritonitis Father: not known
Glu211lys	
WM	Mother: 75 years, heart failure, aortic stenosis Father: 76 years, myocardial infarction

reverse primer for the detection of the ile113thr mutation in exon 4.

SINGLE STRAND CONFORMATION
POLYMORPHISM (SSCP) ANALYSIS
SSCP was performed as previously described.⁷

PATIENTS
Blood samples were taken from 57 patients with sporadic ALS, recruited through the Scottish Motor Neurone Disease Register, a prospective, collaborative, population based study which aims at complete case ascertainment and follow up of all patients diagnosed in Scotland since 1989.^{12,13} The subjects were interviewed for the purposes of a case control study and were classified as sporadic if either patient or carer or both was unable to recall a family history of ALS, or any other form of motor neurone disease at the time of interview in 1991/1992. This classification was made “blindly” before discovery of SOD1 mutations. In patients who were subsequently shown to have mutations, further enquiries were made by contacting GPs, inspecting the death certificates of the parents and grandparents, and, in one case (WM), reinterviewing surviving family members. All four sporadic parents with mutations had a combination of upper and lower motor neurone features and fulfilled local diagnostic criteria. The age and cause of death of the parents of these four patients is shown in table 1.

DNA from 10 ALS patients with a family history of the disease was isolated by consultant neurologists during the same period. In many cases DNA was also extracted from trans-formed lymphoblastoid cell lines from Scottish

patients located at the European collection of animal cell cultures (ECCAC), PHLS, Porton Down.

Results
In our initial screening experiments, using SSCP and direct PCR sequencing, we found two separate mutations, ile113thr and glu211lys, in sporadic patients.^{7,14} In the exon 1 glu211lys mutation a *TaqI* site is destroyed, but in the exon 4 mutation, ile113thr, no restriction site is altered. It was therefore necessary to design a restriction generating PCR primer¹⁵ (RG113), which creates a *BstNI* restriction site when ile113thr is present. PCR amplification with RG113 and the reverse exon 4 primer yields a 126 bp product in normal subjects. If the patient DNA is heterozygous for ile113thr, *BstNI* digestion produces 100 bp and 26 bp fragments while the normal allele remains uncut.

In all the other nine previously described mutations restriction sites were altered, allowing for easy screening (table 2). We found one familial patient with glu93arg and one with glu100gly. Three further cases of ile113thr were discovered, each time in patients with a family history of ALS. The most common ALS mutation in the USA, ala4val in exon 1,⁶ was not present in our population.

In the remaining 58 cases each of the five SOD1 exons was amplified by PCR and the products inspected by SSCP. No further mutations were found.

Discussion
The reports of a variety of SOD1 missense mutations in some cases of familial ALS have provided new insights into the pathogenesis of this fatal neurodegenerative disorder. Our discovery of specific ile113thr SOD1 mutations in three unrelated ALS patients whose disease was apparently sporadic, and subsequent detection of a glu211lys mutation in another sporadic case, suggested either that new mutations in the SOD1 gene were comparatively frequent or that the familial nature of ALS was often disguised. The latter seems more probable, given that the three of the four sporadic cases had identical nucleotide changes and that ALS has late onset and rapid progression to death. In order to try and resolve this issue we have

Table 2 Restriction fragments generated by specific SOD1 mutations and incidence of mutations in patient cohort

Exon	Mutation	Enzyme	PCR product (bp)	Restriction fragments (bp)		ALS patients	
				Normal	Mutant*	Sporadic (n = 57)	Familial (n = 10)
1	Ala4Val	– <i>HaeIII</i>	157	71 36 26 24	71 50 36 26 24	0	0
	Glu21Lys	– <i>TaqI</i>	157	109 48	157 109 48	1	0
2	Leu38Val	+ <i>MaeIII</i>	207	153 54	78 75 153 54	0	0
	Gly41Ser	– <i>HaeIII</i>	207	118 89	207 118 89	0	0
4	Gly41Asp	– <i>HaeIII</i>	207	118 89	207 118 89	0	0
	Gly85Arg	+ <i>HhaI</i>	214	214	185 25 214	0	0
	Gly93Arg	+ <i>Sau3A</i>	214	135 79	44 35 135 79	0	0
	Glu100Gly	– <i>MboII</i>	214	88 68 58	156 88 68 58	0	1
	Asp101Asn	– <i>HinII</i>	214	143 71	214 143 71	0	0
	Ile113Thr	+ <i>BstNI</i>	126	126	100 26 126	3	3
5	Leu144Ser	+ <i>XmnI</i>	224	224	117 107 224	0	0

+ and – signs indicate creation and abolition of restriction sites, respectively
* Mutation specific restriction fragments are in italics.

now undertaken a population based survey of SOD1 mutations in a series of 67 patients collected by the Scottish Motor Neurone Disease Register. To achieve this we amplified each of the five exons of the gene and subjected the products to SSCP analysis. Since all SOD1 base changes detected to date have been missense mutations in exons, the technique has considerable power. It will not, of course, pick up mutations in introns or in the promoter regions. In addition, we examined all our uncharacterised cases for 11 of the 20 reported mutations, using specific restriction enzyme digestion.

No new mutations were discovered in this survey. One case each of the previously reported gly93arg and glu100gly mutations in exon 4 were detected, each time in patients with a clear family history of ALS. However, it is significant that we did detect three further examples of the ile113thr mutation, this time in familial cases. This suggests, but does not prove, that our sporadic ile113thr cases are part of an extended family in which the mutation is segregating. The relatively early deaths of the parents of the probands, together with illegitimacy in the families, make this difficult to prove. However, it is noteworthy that six of the nine SOD1 mutations in this Scottish cohort have involved the same nucleotide change in exon 4. Other investigators have observed that the average age of patients with the ile113thr mutation is 61.2 years, SD 12.9 (n=5), with mean survival of 1.6 years, SD=10 (n=4) (R H Brown Jr, personal communication). Further population based studies should give a clearer idea of the frequency and penetrance of this mutation.

Deng *et al*⁶ have mapped several of their published mutations onto the crystallographic structure of human SOD1. They hypothesise that glu100gly, by virtue of its position in the molecule and the introduction of a charge change, would destabilise the SOD1 structure. Similarly, mutation at gly93, one of four conserved gly residues at which mutations occur, would also have a destabilising effect. Ile113, on the other hand, participates in intrachain hydrogen bonding between SOD1 monomers. Thus ile113thr would adversely affect the structural integrity of the active dimeric SOD1 enzyme.

The failure of ourselves and others⁶ to find mutations around the active site of SOD (encoded by exon 3) strengthens the view that SOD mutations exert their effect in ALS by structural destabilisation and reduction in activity rather than as a result of a totally inactive enzyme. This has been borne out by SOD1 assays on red blood cells from patients carrying a variety of SOD1 mutations.⁶

Scanning all five SOD1 exons by SSCP and screening for specific published mutations in exons 1, 2, 4, and 5 showed mutations in five of the 10 Scottish familial cases. Siddique *et al*² showed linkage to chromosome 21 markers

in only 55% of their families, indicating a degree of genetic heterogeneity in ALS. It is possible that our other five families are unlinked to chromosome 21 and hence to SOD1.

In the collaborative study by Rosen *et al*³ involving 150 ALS families from Boston, Chicago, and Montreal, the ile113thr mutation was only detected in two (1%). In contrast, in our population the frequency is 9% (6/67). This has implications for the local management of both sporadic and familial ALS. Counselling is necessary for those who may produce information about the genetic nature of ALS in their family by the finding of a mutation. Asymptomatic family members who are at risk may seek predictive testing. It is vital that the lessons learned from the Huntington's disease programmes are not neglected,^{16,17} and that adequate time for informed reflection is given before such results are produced. In addition, caution must be urged in the interpretation of results, since little is known about the penetrance and expressivity of SOD1 mutations.

We thank Drs A Chancellor and J de Belleruche for help in collecting samples. This study was supported by a grant from the Scottish Motor Neurone Disease Association.

- Mulder LW, Kurland LT, Offord KP, Beard CM. Familial adult motor neuron disease: amyotrophic lateral sclerosis. *Neurology* 1986;36:511-17.
- 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;324:1381-4.
- 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.
- Halliwell B, Gutteridge JMC. Oxygen radicals and the nervous system. *Trends Neurosci* 1985;8:22.
- Levanon D, Lieman-Hurwitz J, Dafni N, *et al*. Architecture and anatomy of the chromosomal locus in human chromosome 21 encoding the Cu/Zn superoxide dismutase. *EMBO J* 1985;4:77-84.
- Deng HX, Hentati A, Tainer JA, *et al*. Amyotrophic lateral sclerosis and structural defects in Cu, Zn superoxide dismutase. *Science* 1993;261:1047-51.
- Jones CT, Swingler RJ, Brock DJH. Identification of a novel SOD1 mutation in an apparently sporadic amyotrophic lateral sclerosis patient and the detection of Ile113Thr in three others. *Hum Molec Genet* 1994;3:649-50.
- Jones CT, Shaw PJ, Chari G, Brock DJH. Identification of a novel exon 4 SOD1 mutation in a sporadic amyotrophic lateral sclerosis patient. *Mol Cell Probes* 1994;8:329-30.
- Elshafey A, Lanyon WG, Connor JM. Identification of a new missense mutation in exon 4 of the Cu/Zn superoxide dismutase (SOD1) gene in a family with amyotrophic lateral sclerosis. *Hum Molec Genet* 1994;2:363-4.
- Ogasawara M, Matsubara Y, Narisawa K, *et al*. Mild ALS in Japan associated with novel SOD mutation. *Nature Genet* 1993;5:323-4.
- Herskowitz I. Functional inactivation of genes by dominant negative mutations. *Nature* 1987;329:219-22.
- Chancellor AM, Swingler RJ, Fraser H, Warlow CP. The Scottish Motor Neurone Disease Register: a prospective study of adult motor neuron disease in Scotland. Methodology, demography and clinical features of incident cases in 1989. *J Neurol Neurosurg Psychiatry* 1992;55:536-41.
- Chancellor AM, Fraser H, Swingler RJ, Holloway S, Warlow CP, for the Scottish Motor Neurone Research Group. Clinical heterogeneity of familial neuron disease: report of 11 pedigrees from a population-based study in Scotland. *J Neurol Sci* 1994;123:75-6.
- Jones CT, Brock DJH, Chancellor AM, *et al*. Cu/Zn superoxide dismutase (SOD1) mutations and sporadic amyotrophic lateral sclerosis. *Lancet* 1993;342:1050-1.
- Haliassos A, Chamel JC, Tesson L, *et al*. Modification of enzymatically amplified DNA for the detection of point mutations. *Nucleic Acids Res* 1989;17:3606-12.
- Simpson SA, Dean JCS, Hailes NE. Genetic prediction of adult onset disease. *BMJ* 1994;308:535.
- Simpson SA, Harding AE. Predictive testing for Huntington's disease: after the gene. *J Med Genet* 1993;30:1036-8.