Brief papers

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

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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.

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

- exon 3: 3A-5' CTTCCTTGATTAAATAGGCTG 3', 3B-5' AGGCCCATATTTGAGTG 3'
- exon 5: 5A-5' GTGATTACTTGACAGCCAAAAG 3', 5B- 5' CAGGATACATTTCATACAGCTAG 3'

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

The SOD1 gene comprises five small exons5 making it ideal for mutation screening. Since the initial report,6 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.

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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

* Required field


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

| Exon | Mutation | Enzyme | PCR product (bp) | Restriction fragments (bp) | Mutant* | ALS patients 
|------|----------|--------|------------------|---------------------------|---------|----------------- 
|      |          |        |                  |                           |         | Sporadic (n = 57) | Familial (n = 10) |
| 1    | Ala4Val  |        |                  | 71 36 26 24              | 71 59 36 26 24             | 0      | 0               |
|      | Glu21Lys |        |                  | 109 48                    | 157 109 48                 | 0      | 0               |
| 2    | Leu38Val |        |                  | 153 94                    | 78 75 153 54               | 0      | 0               |
|      | Gly41Ser |        |                  | 118 89                    | 207 118 89                 | 0      | 0               |
| 3    | Gly21Asp |        |                  | 118 89                    | 207 118 89                 | 0      | 0               |
| 4    | Gly28Arg |        |                  | 135 79                    | 44 35 135 79               | 0      | 0               |
|      | Gly93Arg |        |                  | 135 79                    | 44 35 135 79               | 0      | 0               |
|      | Gly105Gly|        |                  | 88 68 58                  | 156 88 68 58               | 0      | 0               |
|      | Asp101Asn|        |                  | 143 71                    | 214 143 71                 | 0      | 1               |
| 5    | Ile113Thr|        |                  | 126 126                   | 106 26 126                 | 3      | 3               |

* and — signs indicate creation and abolition of restriction sites, respectively

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 glu21lys 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

Results

In our initial screening experiments, using SSCP and direct PCR sequencing, we found two separate mutations, ile113thr and glu21lys, in sporadic patients.7 In the exon 1 glu21lys mutation a Tagl 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 1,4 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.

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

**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) | Mutant* | ALS patients |
|------|----------|--------|------------------|---------------------------|---------|-------------- 
|      |          |        |                  |                           |         | Sporadic (n = 57) | Familial (n = 10) |
| 1    | Ala4Val  |        |                  | 71 36 26 24              | 71 59 36 26 24             | 0      | 0               |
|      | Glu21Lys |        |                  | 109 48                    | 157 109 48                 | 0      | 0               |
| 2    | Leu38Val |        |                  | 153 94                    | 78 75 153 54               | 0      | 0               |
|      | Gly41Ser |        |                  | 118 89                    | 207 118 89                 | 0      | 0               |
| 3    | Gly21Asp |        |                  | 118 89                    | 207 118 89                 | 0      | 0               |
| 4    | Gly28Arg |        |                  | 135 79                    | 44 35 135 79               | 0      | 0               |
|      | Gly93Arg |        |                  | 135 79                    | 44 35 135 79               | 0      | 0               |
|      | Gly105Gly|        |                  | 88 68 58                  | 156 88 68 58               | 0      | 0               |
|      | Asp101Asn|        |                  | 143 71                    | 214 143 71                 | 0      | 1               |
| 5    | Ile113Thr|        |                  | 126 126                   | 106 26 126                 | 3      | 3               |

* 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 the same 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. 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 others 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. 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. 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. 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 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.

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