Brief papers

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, ileI13thr, 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 leukocytes 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”), and exon 4 (set “a”) were as described. The primers used for exons 3 and 5 were:

exon 3:

3A-5’ CTTCTTCTTATAATAGGCTG 3’
3B-5’ AGGCACATATTTACAAAGTATG 3’

exon 5:

5A-5’ GTGATTACTTGACAGCCCAAG 3’
5B-5’ CAGGATACATTTCATAAGCTAG 3’

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

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

<table>
<thead>
<tr>
<th>Exon</th>
<th>Mutation</th>
<th>Enzyme</th>
<th>PCR product (bp)</th>
<th>Restriction fragments (bp)</th>
<th>ALS patients</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Normal</td>
<td>Mutant*</td>
<td>Sporadic (n=57)</td>
</tr>
<tr>
<td>1</td>
<td>Ala4Val</td>
<td>HaIII</td>
<td>157</td>
<td>71 36 26 24</td>
<td>0</td>
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<tr>
<td>2</td>
<td>Glu211ys</td>
<td>TaqI</td>
<td>157</td>
<td>109 94 48</td>
<td>1</td>
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<tr>
<td></td>
<td>Lec38Val</td>
<td>MstIII</td>
<td>207</td>
<td>153 54 153 54</td>
<td>0</td>
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<tr>
<td></td>
<td>Gly41Ser</td>
<td>HaIII</td>
<td>207</td>
<td>118 89 207 118 89</td>
<td>0</td>
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<tr>
<td></td>
<td>Gly41Asp</td>
<td>HaIII</td>
<td>207</td>
<td>118 89 207 118 89</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Gly85Arg</td>
<td>HhaI</td>
<td>214</td>
<td>214 185 214 185 214</td>
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<tr>
<td></td>
<td>Gly93Arg</td>
<td>Sau3A</td>
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<td>135 79 44 135 79</td>
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<td>Asp101Asn</td>
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<td>Ile133Thr</td>
<td>BrNI</td>
<td>126</td>
<td>126 100 26 126</td>
<td>3</td>
</tr>
</tbody>
</table>

* 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. 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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