The spectrum of Notch3 mutations in 28 Italian CADASIL families


Background: Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a cause of hereditary cerebrovascular disease. It results from mutations in the Notch3 gene, a large gene with 33 exons. A cluster of mutations around exons 3 and 4 was originally reported and limited scanning of these exons was suggested for the diagnosis in most cases.

Objective: To report Notch3 mutation analysis in 28 unrelated Italian CADASIL families from central and south Italy.

Results: The highest rate of mutations was found in exon 11 (21%) and only 18% of mutations were in exon 4. This may be related to the peculiar distribution of Notch3 mutations in the regions of origin of the families.

Conclusions: The results suggest that limited scanning of exons 3 and 4 is inadvisable in CADASIL cases of Italian origin.

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is now recognised to be a cause of hereditary cerebrovascular disease. It results from mutations in Notch3, a gene encoding a transmembrane protein involved in cell signalling and cell differentiation. All the mutations described up to now result in a gain or loss of one cysteine residue in one of 34 epidermal growth factor-like repeats. The clinical spectrum of the disease is broad, even in the same family. The most frequent manifestations include one or more of the following: recurrent ischaemic episodes (transient ischaemic attacks or stroke), migraine with aura, cognitive decline, and psychiatric changes. The white matter abnormalities detected by magnetic resonance imaging (MRI) are strongly suggestive but often indistinguishable from other neurological disorders such as multiple sclerosis, lacunar stroke with leukoaraoisias, and adult onset leukoencephalopathies. This makes the clinical diagnosis of CADASIL difficult and implies that it may be underestimated in the wider population with MRI evidence of diffuse white matter abnormalities.

The hallmark of the disease is the presence of deposits of granular osmiophilic material (GOM) in the small vessels. Electron microscopic screening of skin arterioles for GOM was the first diagnostic method proposed but it is not sufficiently sensitive (up to 50% false negatives (Markus et al6 and our own unpublished data). Diagnosis of CADASIL is therefore confirmed by demonstration of mutations in the Notch3 gene. Complete Notch3 gene mutation analysis is expensive and time consuming because of the large number of exons, and it is therefore unsuitable for routine diagnosis. Although CADASIL has a broad mutational spectrum, clustering of mutations around exon 3 and especially exon 4 was originally reported in about 70% of cases and it was subsequently suggested that scanning should be limited to these exons for quick identification of suspected cases. However, further studies of the spectrum of Notch3 gene mutations in a geographically localised population revealed significant divergence from this pattern and suggested the need to examine additional exons (numbers 5, 6, 11, and 18), largely dependent on the population screened. Here we describe the mutation pattern of the Notch3 gene in 28 Italian families with CADASIL from central and southern Italy.

METHODS

Notch3 gene mutation screening of exons 2 to 23 (starting with exons 4 and 3) by denaturing high performance liquid chromatography (DHPLC) followed by direct sequencing was carried out in 28 new index cases, belonging to unrelated consecutively diagnosed families from central (13 cases) and southern Italy (15 cases), with clinical and MRI diagnosis of CADASIL. Informed consent was obtained in all cases. The analysis was carried on in two laboratories, Siena and Piano Lago di Mangone (Cosenza), which are the referral centres in central and southern Italy, respectively, for the diagnosis of CADASIL, in the framework of the Italian CADASIL group. The prevalence of mutations in different exons was determined and the relation to geographical origin analysed.

Genomic DNA was extracted from buffy coat previously prepared from peripheral blood. Polymerase chain reaction (PCR) was undertaken with primers (comprising intron–exon boundaries) specific for exons of the Notch3 gene. PCR reactions were carried out in 50 μl reaction volumes containing 130 ng genomic DNA, 20 pmol forward and reverse primers, 0.2 mM dNTPs, 2U DNA polymerase (Optimase, Transgenomic, Crewe, UK), 1.5 mM MgSO4, and 1X Optimase buffer using a DNA thermal cycler (model 9600; Perkin Elmer, Foster City, California, USA). The mixtures were amplified as recommended by the Optimase protocol with annealing temperature for each primer couple. PCR products were analysed by DHPLC using the Wave system (Transgenomic Inc, Omaha, Nebraska, USA). When a variant pattern was detected by DHPLC, the sample was sequenced using the ABI Prism Big Dye Terminator cycle sequencing ready reaction kit (Perkin Elmer).

RESULTS

Eighteen different mutation sites were detected in the 28 index cases, and seven novel mutations were identified: one deletion in each of exons 3' and 4' and five missense mutations (three in exon 8 and one in each of exons 6 and 10) (table 1).

Abbreviations: CADASIL, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; DHPLC, denaturing high performance liquid chromatography; GOM, granular osmiophilic material
Five families had mutations in exon 4 (17.8%) and exon 3 (17.8%), six families in exon 11 (21.4%), four families in exon 8 (14.3%), three families in exon 6 (10.7%), two families in exon 19 (7.1%), and one family each in exons 10, 20, and 22 (3.6%). All families with mutation in exon 4 (five of five) and the most of those with mutation in exon 11 (four of six) were from central Italy. Otherwise, mutations in exon 3 (five of five) and exon 8 (four of four) were detected only in families from southern Italy. All cases with involvement of exon 11 carried the same C1897T mutation.

**DISCUSSION**

This is the first mutation spectrum analysis in a significant number of unrelated Italian families with CADASIL. Surprisingly, contrary to previous reports in different series of patients, exon 4 was not the most common site of mutation, the proportion being less than 18%. Taken together, mutations in exon 4 and 3 accounted for only 36% of CADASIL cases, less than half the figure reported in the original French series and, more recently, in the British one. On the other hand, mutations in exon 11, the most common in our analysis, were not found in any of the 48 British cases and were rarely found in other less homogeneous series. We also detected a substantial number of mutations in exon 8 (14%) and exon 6 (11%). Moreover, the geographical distribution of some mutations is noteworthy: exon 4 mutations were only found in families from central Italy, whereas mutations in exons 3 and 8 were detected only in families from the south. To our knowledge, only a few Italian CADASIL families have been reported. Mutations were found in exon 4 in five of five and in exon 6.

In all but two cases, the present analysis showed missense mutations, five of which were novel: three in exon 8 and one each in exons 6 and 10 (table 1). No mutation has previously been reported in exon 10. Interestingly, the same C1897T mutation involved exon 11 in all cases (six of six), irrespective of geographical origin. The recurrence of this rare mutation in six apparently unrelated families is striking. However, a founder effect cannot be ruled out. Cases 5 and 10 (table 1) carried two novel deletions in the Notch3 gene adding to the few (only four) mutations of this type already reported. The deletion on exon 3 did not involve a cysteine residue.

CADASIL is an increasingly recognised hereditary cerebrovascular pathology leading to progressive neurological handicap. The wide variability in clinical presentation and evolution, the apparent absence of inheritance in many cases, and the substantially non-specific pattern of MRI lesions, at least in the early stages, make clinical diagnosis difficult. The problem of diagnostic screening is a crucial point and the subject of international debate. That the disease is not only underestimated but also occurs worldwide has become evident since genetic testing became widely available. The peculiar distribution of Notch3 gene mutations emerging from the first population studies suggests that a different mutation analysis approach is needed from country to country, and as the present findings intimate, perhaps also in different parts of the same country.

Our results strongly suggest that the spectrum of CADASIL mutations should be analysed in different populations and that the regional origin of families should be taken into account in planning the expensive and time consuming molecular screening protocol. In Italy scanning limited to exons 3 and 4 would have confirmed the diagnosis in only one third of cases.

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**Table 1** The spectrum of Notch3 mutations found in 28 Italian families

<table>
<thead>
<tr>
<th>Family</th>
<th>Origin</th>
<th>Exon</th>
<th>Mutation site</th>
<th>Amino acid change</th>
<th>Proportion with exon</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>South</td>
<td>3</td>
<td>C406T</td>
<td>Arg110Cys</td>
<td>17.8%</td>
</tr>
<tr>
<td>2</td>
<td>South</td>
<td>3</td>
<td>C406T</td>
<td>Arg110Cys</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>South</td>
<td>3</td>
<td>C346T</td>
<td>Arg90Cys</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>South</td>
<td>3</td>
<td>C346T</td>
<td>Arg90Cys</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>South</td>
<td>3</td>
<td>Deletion from C341</td>
<td>to G352* to Gly91</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Central</td>
<td>4</td>
<td>C499T</td>
<td>Arg141Cys</td>
<td>17.8%</td>
</tr>
<tr>
<td>7</td>
<td>Central</td>
<td>4</td>
<td>T625A</td>
<td>Cys183Ser</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Central</td>
<td>4</td>
<td>C697T</td>
<td>Arg207Cys</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Central</td>
<td>4</td>
<td>C697T</td>
<td>Arg207Cys</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Central</td>
<td>4</td>
<td>Deletion from T312</td>
<td>Frame shift to G316* to G312</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>South</td>
<td>6</td>
<td>C1072T</td>
<td>Arg322Cys</td>
<td>10.7%</td>
</tr>
<tr>
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<td>6</td>
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<td>Arg322Cys</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Central</td>
<td>6</td>
<td>T1090C*</td>
<td>Cys338Arg</td>
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</tr>
<tr>
<td>14</td>
<td>South</td>
<td>8</td>
<td>G1361A*</td>
<td>Cys428Tyr</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>South</td>
<td>8</td>
<td>T1360C*</td>
<td>Cys428Tyr</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>South</td>
<td>8</td>
<td>T1393A*</td>
<td>Cys440Ser</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>South</td>
<td>8</td>
<td>C1423T</td>
<td>Arg449Cys</td>
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<tr>
<td>18</td>
<td>South</td>
<td>10</td>
<td>G1660T*</td>
<td>Gly528Cys</td>
<td>3.6%</td>
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<tr>
<td>19</td>
<td>Central</td>
<td>11</td>
<td>C1897T</td>
<td>Arg607Cys</td>
<td>21.4%</td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>21</td>
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<td>11</td>
<td>C1897T</td>
<td>Arg607Cys</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Central</td>
<td>11</td>
<td>C1897T</td>
<td>Arg607Cys</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Central</td>
<td>11</td>
<td>C1897T</td>
<td>Arg607Cys</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>South</td>
<td>11</td>
<td>C1897T</td>
<td>Arg607Cys</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Central</td>
<td>19</td>
<td>C3094T</td>
<td>Arg1006Cys</td>
<td>7.1%</td>
</tr>
<tr>
<td>26</td>
<td>Central</td>
<td>19</td>
<td>C3094T</td>
<td>Arg1006Cys</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>Central</td>
<td>20</td>
<td>C3304T</td>
<td>Arg1076Cys</td>
<td>3.6%</td>
</tr>
<tr>
<td>28</td>
<td>South</td>
<td>22</td>
<td>C3769T</td>
<td>Arg1231Cys</td>
<td>3.6%</td>
</tr>
</tbody>
</table>

*New mutation.
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