Yield of Screening for CADASIL Mutations in Lacunar Stroke and Leukoaraiosis

Yanbin Dong, PhD; Ahamad Hassan, MRCP; Zhongyi Zhang, MSc; Dionne Huber, MSc; Chrysoula Dalageorgou, BSc; Hugh S. Markus, FRCP

Background and Purpose—Cerebral autosomal dominant arteriopathy subcortical infarcts and leukoencephalopathy (CADASIL) is a monogenic disorder typified by early onset lacunar strokes, subcortical dementia, psychiatric disturbances, and migraine. Mutations in the Notch3 gene are responsible. Atypical phenotypes have been recognized, and the disease is probably underdiagnosed in the wider stroke population. Therefore, we determined the yield of screening for Notch3 mutations in lacunar stroke with or without leukoaraiosis.

Methods—Two hundred eighteen consecutive patients were studied. All had brain and carotid imaging. Polymerase chain reaction–single-stranded conformational polymorphism analysis was used to screen exons 3, 4, 5, and 6 of the Notch3 gene for mutations and polymorphisms.

Results—A single mutation in exon 4 (C697T) was identified in a young patient, giving an overall carrier frequency of 0.05% (95% CI, 0.0 to 2.0). For patients with onset of lacunar stroke at ≤65 years and leukoaraiosis, the yield was 2.0% (95% CI, 0.4 to 10.9).

Conclusions—Notch3 mutations are rare in patients with typical strokes due to cerebral small-vessel disease. In the absence of classic features suggestive of CADASIL, screening for Notch3 mutations has a low yield. (Stroke. 2003;34:203-206.)

Key Words: CADASIL ■ genetic screening ■ lacunar infarction ■ leukoaraiosis
CADASIL cases. Therefore, we screened these 4 exons in this study.

**Subjects and Methods**

**Study Population**

Two hundred eighteen consecutive patients with lacunar stroke defined by modified Trial of Org 10172 (TOAST) criteria were studied. All were referred to a South London cerebrovascular service between March 1995 and June 2001. Exclusion criteria were as follows: carotid or vertebral stenosis >50%, cortical infarcts, lacunar infarct >1.5 cm in diameter, and potential cardiac source of embolism. All subjects gave informed consent for the study, which was approved by the local research ethics committee. A positive family history was recorded when 1 or more first-degree relatives had been reported to be affected by stroke. Patients were considered to have leukoaraiosis if they had a periventricular leukoencephalopathy score of 3 on CT/MRI defined according to the Fazekas scale.

Associations between alleles and phenotypic characteristics were determined with the use of χ² statistics. The study was powered to detect a mutation carrier frequency of 1% (β>80%, P=0.05).

**Genotyping**

All patients were screened for mutations in exons 3, 4, 5, and 6 by polymerase chain reaction followed by single-stranded conformational polymorphism (SSCP), with the use of previously published primers. Abnormal SSCP migration patterns were then characterized by direct sequencing. Previously published Notch mutations were also identified with the use of restriction digestion enzyme assays. DNA with known Notch3 mutations, determined by direct sequencing, was used as positive internal control for SSCP and restriction digestion. The positive controls included 3 mutations in exon 3 (T304C, C346T, and C406T), 7 mutations in exon 4 (C499T, C535T, C583T, C622T, T625C, T658A, and C697T), and 1 mutation each in exon 5 (T829C) and exon 6 (C1072T). Two polymorphisms (C381T in exon 3 and G684A in exon 4) were also used as controls.

**Results**

Patient characteristics are shown in the Table. There were 120 patients with stroke onset at ≤65 years, 48 of whom had moderate or severe leukoaraiosis. There were 24 patients with stroke onset at ≤50 years, 9 of whom had moderate or severe leukoaraiosis. A positive family history was documented in one third of patients, but there were no large kindreds with multiply affected family members. DNA was amplified for all 4 exons in 210 individuals. In the remaining 8 cases, genotyping was successful in at least 1 exon. All control mutations and polymorphisms were successfully identified by both SSCP analysis and restriction digestion enzyme assays.

A single Notch3 mutation was identified, located within exon 4: C697T. The overall mutation carrier frequency was 0.05% (95% CI, 0.0 to 2.0). For subjects with lacunar stroke and leukoaraiosis, the frequency was 2.0% (95% CI, 0.4 to 10.9) for those with disease onset at ≤65 years and 11.1% (95% CI, 2.5 to 44.5) for those with disease onset at ≤50 years. The patient with positive findings was a 38-year-old man of African ethnicity who presented with dysarthria and hemiparesis. This patient was a smoker and had a fasting cholesterol level of 8.3 mmol/L and an erythrocyte sedimentation rate of 43. The MRI revealed multiple lacunar infarcts and leukoaraiosis; the working diagnosis had been either cerebral vasculitis or sporadic lacunar stroke.

Three polymorphisms were identified. The previously reported polymorphisms C381T (exon 3) and G684A (exon 4) were present in 57 (26.6%) and 43 cases (19.7%), respectively, and a rare novel polymorphism C738T (exon 4) was present in 2 cases (0.9%). No association was found between the 2 common polymorphisms and leukoaraiosis (C381T: χ²=0.98, P=0.32; G648A: χ²=3.24, P=0.72) or a history of migraine (C381T: χ²=0.1, P=0.75; G648A: χ²=0.76, P=0.78).

**Discussion**

There are no previous published studies in which a well-phenotyped population of patients with lacunar stroke has been screened for CADASIL. We found the overall frequency of CADASIL mutations in lacunar stroke to be <0.5%. When only younger patients who had radiological leukoaraiosis were considered, the frequency rose to 2% and 11% for individuals with disease onset at ≤65 and ≤50 years, respectively. Wang and colleagues used a restriction enzyme–based technique to perform Notch3 screening in 70 patients with all types of sporadic ischemic stroke and reported no mutations in exons 3 and 4. Since lacunar stroke constitutes a quarter of ischemic strokes, this study would have screened a small number of patients with the actual phenotype caused by CADASIL.

A number of polymorphisms were identified in this study, 2 of which are known, and their frequency was in agreement with a previous report. None of the polymorphisms encoded amino acid changes, nor were they associated with any clinical characteristics. It is therefore likely that these variants are nonfunctional. Two studies failed to find an association between Notch3 polymorphisms and sporadic stroke. 

<table>
<thead>
<tr>
<th>Clinical Characteristics of the 218 Individuals Screened for Notch-3 Mutations and Polymorphisms</th>
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</thead>
<tbody>
<tr>
<td><strong>Median age (range)</strong></td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
</tr>
<tr>
<td>White</td>
</tr>
<tr>
<td>African/Afro-Caribbean</td>
</tr>
<tr>
<td>Oriental</td>
</tr>
<tr>
<td>Indian subcontinent</td>
</tr>
<tr>
<td><strong>Male gender</strong></td>
</tr>
<tr>
<td><strong>Hypertension</strong></td>
</tr>
<tr>
<td><strong>Diabetes mellitus</strong></td>
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<tr>
<td><strong>Current or ex-smoker</strong></td>
</tr>
<tr>
<td><strong>Hypercholesterolemia</strong></td>
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<tr>
<td><strong>Myocardial infarction</strong></td>
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<td><strong>Migraine</strong></td>
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<tr>
<td><strong>Family history of stroke</strong></td>
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<tr>
<td><strong>Family history of stroke ≤65 years</strong></td>
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<td><strong>Age first stroke ≤50</strong></td>
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<td><strong>Age first stroke ≤65 years</strong></td>
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<tr>
<td><strong>Recurrent strokes</strong></td>
</tr>
<tr>
<td><strong>Leukoaraiosis (Fazekas scale ≥3)</strong></td>
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</tbody>
</table>

Values are expressed as proportions (%) for categorical data.

†Family history was unavailable in 14 cases because of adoption or lost contact with relatives.
Our population with lacunar stroke had risk factor profiles similar to those of previous populations of patients with this stroke subtype, with hypertension being present in 76%. Although we did not identify any large kindreds with multiply affected members, a third of our patients reported a positive family history. However, a family history of stroke is frequently documented in patients with presumed sporadic ischemic stroke and has been taken as evidence for polygenic risk factors. The proportion with a family history in this study was very similar to that found in our stroke database of all ischemic stroke subtypes.

We screened 4 exons for mutations according to a recent study, mutations in these exons account for approximately 90% of CADASIL cases in our population. This was a pragmatic approach when the large number of samples analyzed and the cost of screening are considered. It is plausible that the disease frequency could increase if the remaining exons were analyzed. We used SSCP to screen for mutations, which were then confirmed by sequencing. There is a small risk of SSCP missing mutations of perhaps up to 10%. However, we were able to detect all of our positive controls, the majority of which had been previously identified by sequencing rather than by SSCP. This suggests that it is unlikely that we missed mutations in our study.

In most patients with CADASIL, the diagnosis is relatively clear in those with early onset lacunar stroke, often complex migraine, and a typical MRI scan. In the majority of cases there is a clear autosomal pattern of inheritance, with stroke or dementia occurring in relatives at a young age. With more widespread genetic testing, atypical CADASIL phenotypes have also been described. In particular, patients have presented with their first lacunar stroke after the age of 65 years and in the absence of other clinical features. Although this is a recognized presentation, our study suggests that this is rare on a population level. Screening may be worthwhile in younger patients with lacunar stroke who also have radiological leukoaraiosis, but in older individuals or those without leukoaraiosis it is not worthwhile.

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References
apparent that the disease is underdiagnosed. This may suggest that more systematic screening for CADASIL mutations is indicated, at least in the settings in which clinical features of the disease spectrum are common. Groups of patients or subjects to be screened may include the following: patients with vascular dementia, which represents one fourth of total dementia; patients with lacunar stroke, which accounts for one fifth of total first-ever stroke; and subjects with incidental white matter changes on brain imaging, which are highly prevalent in the general population, although both the prevalence and severity of these nonselective changes are definitely age related. A family history of stroke and stroke-associated abnormalities is common among all these patients/subj ects. The recent observation of increased homocysteine levels in patients with CADASIL suggests the intriguing hypothesis that the phenotypic expression of CADASIL might be modulated by additional genetic or acquired factors. Thus, a more extensive search for CADASIL mutations might also contribute to elucidation of the pathophysiology of the disease syndrome.

The question of the extent to which it is worthwhile to screen for CADASIL is a controversial issue. The study of Dong et al in the accompanying article begins to explore this point. They evaluated the yield of systematically screening for CADASIL mutations in a well-defined group of 218 patients with lacunar stroke consecutively examined in a stroke service. The screening was limited to 4 (exons 3, 4, 5, 6) of the 33 exons of the Notch3 gene, which, as mentioned by the authors, have been estimated to be the site of the mutation in 90% of CADASIL cases in a British population. Among the 218 patients, the authors were able to detect only 1 patient with a mutation in exon 4 of the Notch3 gene, accounting for 0.05% of the whole group. The positive case was a 38-year-old man of African ethnicity, presenting with dysarthria and hemiparesis. He was a smoker, had high cholesterol levels, and showed multiple lacunar infarcts and white matter changes on brain MRI. When the analysis was restricted to those with disease onset at ≤50 years, the frequency of CADASIL-positive cases rose to 11%. We agree with the authors’ conclusion that for now, on a population level, screening may be worthwhile in younger patients with lacunar stroke who also have white matter changes, but in older patients or in those without these changes it is not cost-effective. The proportion of genetically defined cases could rise, extending the analysis to the whole gene. However, unless descriptions of new mutations increase to a great extent, complete analysis is not recommended when the expense and time-consuming nature of the procedure are considered.

The availability of predictive clinical and laboratory tests, including, for instance, selective MRI studies or skin biopsy with histopathology studies of skin small-vessel wall, possibly corroborated by immunostaining with antibodies specific for Notch3, could be easily and widely applied before the genetic determination. This might improve the yield of genetic analyses, enlarging the indications for screening. However, before they are used systematically, both positive and negative predictive values of pregeneics tests should be reliably estimated. To achieve this goal, with the degree of the phenotypic variance taken into account, large samples of genetically determined cases to be used as the gold standard would be needed. The availability of a number of validated first-level clinical and laboratory markers could lead to the design, with the use of a probability statistical model, of an algorithm that may help in the decision of whether genetic analyses should be performed. Wide collaborations are mandatory for these tasks to be successfully accomplished.

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