Clinical Spectrum of CADASIL and the Effect of Cardiovascular Risk Factors on Phenotype

Study in 200 Consecutively Recruited Individuals

Poneh Adib-Samii, MRCP; Glen Brice, BSc; Roswell J. Martin, FRCP; Hugh S. Markus, FRCP

Background and Purpose—Cerebral Autosomal-Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) is an inherited arteriopathy with clinical features that include recurrent lacunar stroke, migraine, and cognitive impairment. For reasons that remain unclear, there is great variability in the clinical expression of CADASIL, both between and within families. This study examined the clinical phenotype as well as any associations with risk factors and genotype in a large, prospective cohort.

Methods—Two hundred symptomatic individuals from 124 families were recruited as part of a UK prevalence study of CADASIL and were seen subsequently in a national referral clinic. All were assessed by a standardized questionnaire and examination.

Results—Mean age at assessment was 47.7 years and was 33.6 years at symptom onset. Migraine, usually with aura, was the most prevalent feature, affecting 75% of individuals. More than half had a history of stroke, with a mean age at onset of 46 years. Hypertension (odds ratio 2.57, \( P = 0.007 \)) and pack-years of smoking (odds ratio 1.07, \( P = 0.001 \)) were associated with an increased risk of stroke. A history of stroke was a significant risk factor for both dementia and disability. Mutations clustered in exon 4 of the NOTCH3 gene, which contained \( \geq 71.4\% \) of familial mutations. Four previously unreported mutations were found (T697C, C1279T, G1370C, and C1774T). No associations were identified between genotype and clinical phenotype.

Conclusions—Our data suggest that cardiovascular risk factors may modulate the clinical expression of CADASIL. The associations with hypertension and smoking suggest that risk factors should be treated aggressively in patients with CADASIL. (Stroke. 2010;41:630-634.)

Key Words: CADASIL • phenotype • stroke • risk factors • hypertension

Cerebral Autosomal-Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) is an increasingly diagnosed monogenic cause of lacunar stroke. Mutations in the NOTCH3 gene result in a systemic small-vessel arteriopathy with clinical manifestations confined to the central nervous system. Clinical features include recurrent lacunar stroke, migraine, depression, and cognitive impairment progressing to dementia. Magnetic resonance imaging (MRI) shows leukoaraiosis with additional lacunar infarcts in older patients.

Studies to date suggest that the CADASIL phenotype is variable not only between families but also within families. The reason for these differences is unclear, but a role for modulating genes has been proposed. Although early studies suggested that coexistent cardiovascular risk factors were rare, more recent data suggest that these risk factors may modulate disease severity.

There have been few large, prospectively collected, patient series describing the detailed clinical phenotype. In this article, we present the first 200 symptomatic cases seen as part of a UK study. Data were prospectively collected according to standard protocols. As well as describing phenotypic characteristics, we also studied relations between cardiovascular risk factors and clinical features and genotype-phenotype relations.

Subjects and Methods

Participants and Recruitment

The first 200 consecutive subjects with CADASIL reviewed by a UK CADASIL National Referral service were reviewed; the details of and initial recruits to this study have been previously described. They were seen initially as part of a UK prevalence study and subsequently in a national referral clinic. A diagnosis of CADASIL was made when confirmed by direct sequencing of the NOTCH3 gene (193 cases), by skin biopsies showing the presence of granular osmophilic material by electron microscopy, or by typical MRI appearances in combination with a mutation-confirmed family history. Informed consent was gained from all participants, and the study was approved by the local ethics committee.

All participants were prospectively assessed by 1 of 2 consultant neurologists (H.S.M., R.J.M.) who used a standardized questionnaire and examination. The following information was recorded: presence and age at onset of cardiovascular risk factors; presence and age at...
onset of stroke, migraine, psychiatric illnesses, dementia, acute reversible encephalopathy, and seizures; neurologic examination findings; functional dependence as measured by the modified Rankin scale; and the Mini-Mental State Examination score. \(^{11}\)

Hypertension was defined as systolic blood pressure \(>140\) mm Hg, diastolic blood pressure \(>90\) mm Hg, or use of antihypertensive medication. \(^{12}\) Hypercholesterolemia was defined as nonfasting cholesterol \(>5.2\) mmol/L or drug treatment for hypercholesterolemia. \(^{13}\) Smoking history, including total pack-years at the time of assessment, was recorded. Diabetes was defined as a previous diagnosis of diabetes or a fasting blood glucose \(>7.0\) mmol/L. \(^{14}\) The age at onset of risk factors was defined as the age at which treatment was commenced or the age at diagnosis for risk factors detected during the patient study assessment. Homocysteine was measured in serum by competitive immunoassay, and hyperhomocysteinemia was defined as a random homocysteine level \(>5.2\) mmol/L. \(^{15}\) Dementia was defined as a previous diagnosis of dementia made by a neurologist/psychiatrist or a Mini-Mental State Examination score \(<23\), which has been found to be consistent with the Diagnostic and Statistical Manual of Mental Disorders' criteria for dementia. \(^{16}\) A diagnosis of acute reversible encephalopathy was made clinically \(^{16}\) and was attributed to CADASIL when no other cause could be found. Depression was defined as a history of depression for which the individual had received antidepressant drug therapy. Migraine definitions were based on the 2004 International Headache Society Criteria, although it should be noted that these criteria exclude secondary causes of migraine. Functional independence and dependency were defined as Rankin scores of 0 to 2 and 3 to 5, respectively. All original MRI scans were reviewed centrally by a consultant neurologist (H.S.M.) to determine the site and type of any infarcts.

### Genetic Analysis

Two techniques of genetic analysis were performed during the study period, which reflect technological advances. Screening for \(N\)OTCH3 mutations was performed by direct sequencing with an ABI3730 automated sequencer, initially of exons 3 and 4. In mutation-negative cases, single-strand conformation polymorphism of additional exons coding for the extracellular portion of the protein (ie, 2 and 5 to 23) was performed, followed by direct sequencing of any variants identified. More recently, the initial screening strategy involved direct sequencing of exons 3 and 4 with an ABI 3730 automated sequencer followed by, in mutation-negative cases, denaturing high-performance liquid chromatography of exons 5, 6, 8, 11, 18, and 22 and subsequent direct sequencing when variants were identified. Screening these exons has been shown to detect \(>90\%\) of mutations in UK CADASIL patients. \(^9\)

### Skin Biopsies

In a subgroup of patients, skin biopsy was performed. A 6-mm full-thickness or 2- to 3-mm punch biopsies were taken from the anterior abdomen or upper arm, fixed, and examined by electron microscopy for the presence of granular osmophilic material, as previously described. \(^9,10\)

### Statistics

For statistical analyses, SPSS version 16 was used, and probability values \(<0.05\) were considered statistically significant. The relation between cardiovascular risk factors and the presence and age at onset of clinical phenotypes were determined. To determine the effect of risk factors on the age at onset of clinical outcomes, Kaplan–Meier analysis was used, followed by Cox regression to control for cardiovascular risk factors. Binary logistic regression was used to assess associations between risk factors and categorical clinical outcomes and associations between different clinical phenotypes. When a significant association was identified, this was followed by multivariate analyses, covarying for sex and cardiovascular risk factors. On multivariate analyses, the following cardiovascular risk factors were controlled for: age at assessment, sex, hypercholesterolemia, ever-smoker, and hypertension. Diabetes was not included, because it affected only \(4\%\) of the study group.

To determine the relation of phenotype with mutation site, 2 statistical analyses were performed. The logarithm of codon position of the mutated amino acid was taken to normalize the distribution, and then binary logistic regression was used to examine the relation between clinical phenotype and codon site. Second, a \(\chi^2\) analysis was used to compare the expected frequency of clinical outcomes in mutations by codon and exon with expected frequencies. When an expected count of \(<5\) was encountered, Fisher’s exact test was applied. Similarly, \(\chi^2\) statistics were used to determine any significant clustering of clinical phenotypes within families.

### Results

#### Clinical Characteristics and Risk Factor Profiles

Two hundred symptomatic individuals from 124 families were included, and \(86\) (43\%) individuals were male. All were of white ethnicity with the exception of 1 Middle Eastern, 1 black African, and 2 mixed-ethnicity individuals. The mean age at assessment was 47.7 (SD 11.4) years, and the mean age at first symptom onset was 33.6 (SD 14.1) years. The prevalence of clinical features and risk factors at the time of assessment is shown in Table 1.

The frequencies of the first features and first major events of CADASIL are shown in Table 2. First major events were defined as stroke, encephalopathy, seizure, dementia, or other, which included pseudobulbar palsy, spastic paraparesis, and a parkinsonian syndrome. Seventy-three (35.5\%) individuals had not experienced a major event at the time of assessment. The relations between cardiovascular risk factors and clinical features are presented in Table 3.

### Stroke

One hundred two (51.5\%) individuals had a history of stroke, and the mean age at onset was 46 (SD 9.7) years. All strokes were clinically and radiographically lacunar, with the excep-
tion of 1 brainstem hemorrhage in an individual on warfarin therapy and 1 cortical infarct in a 67-year-old female ex-smoker with hypercholesterolemia. Forty-nine (24.5%) individuals had experienced $>1$ stroke. The distribution of age at onset of stroke is shown in Figure 1.

Stroke risk was increased in hypertensive individuals (odds ratio [OR] = 2.57; 95% CI, 1.29 to 5.14; $P = 0.007$), and this trend remained after covarying for age and cardiovascular risk factors (OR = 2.31; 95% CI, 1.06 to 5.05; $P = 0.036$). Stroke was associated with pack-years of smoking (OR = 1.07; 95% CI, 1.03 to 1.12; $P = 0.001$), and this trend also remained after covarying for age and cardiovascular risk factors (OR = 1.05; 95% CI, 1.01 to 1.10; $P = 0.029$). Current smoking status was associated with an earlier age at onset of stroke (hazard ratio = 1.68; 95% CI, 1.05 to 2.67; $P = 0.03$), and this trend remained significant after covarying for cardiovascular risk factors (hazard ratio = 1.79; 95% CI, 1.08 to 2.97; $P = 0.024$) (Figure 1).

There were no associations between other risk factors and stroke, including body mass index, raised cholesterol, or elevated serum homocysteine. A history of migraine with aura was associated with a reduced risk of stroke (OR = 0.56; 95% CI, 0.19 to 0.67; $P = 0.001$), and this trend persisted after covarying for age and risk factors (OR = 0.38; 95% CI, 0.19 to 0.77; $P = 0.007$).

**Migraine**

One hundred fifty (75%) individuals had experienced migraine, of whom 135 (90%) experienced aura. Mean age at onset was 29.4 (SD 12.7) years, and the distribution of age at onset is shown in Figure 1. The most common aura was sensory (n = 102, 75.6%) followed by visual (n = 87, 64.4%), dysphasic (n = 35, 25.9%), and motor (n = 18, 13%). Of those experiencing auras, 83 (61.5%) experienced auras in $>1$ domain. Forty-five (33%) migraineurs with aura reported prolonged auras, defined as lasting $>1$ hour but $<1$ week. There were no cases of migrainous status (aura lasting $>1$ week). Twenty-four (16%) migraineurs reported acute confusion associated with migraine, defined as disorientation in time or place with or without retrograde amnesia.

Female sex was associated with an earlier age at onset of migraine, and this persisted after controlling for cardiovascular risk factors (hazard ratio = 1.45; 95% CI, 1.02 to 2.06; $P = 0.039$). Neither the presence of nor age at onset of migraine was associated with cardiovascular risk factors.

**Encephalopathy**

Twenty-one (10.5%) individuals experienced an episode of reversible encephalopathy, and in 18 (9%), this was the first major event. Mean age at onset was 41.5 (SD 11.4) years. Eight (38.1%) individuals experienced relapses. The maximum number of encephalopathic episodes experienced by 1 individual was 4, with the median interval between relapses of 24.5 months (range, 9 to 120). In all individuals with the exception of 1, the encephalopathy followed a typical migraine with aura, and 5 experienced seizures during the episode. All individuals reported a history of migraine with aura outside the encephalopathic episodes. Neither the pres-

### Table 2. Frequency of First (Presenting) Features and First Major Event for 200 CADASIL Subjects

<table>
<thead>
<tr>
<th>First (Presenting) Feature</th>
<th>First Major Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>Migraine</td>
<td>Stroke</td>
</tr>
<tr>
<td>134 (67%)</td>
<td>39 (19.5%)</td>
</tr>
<tr>
<td>Stroke</td>
<td>96 (48%)</td>
</tr>
<tr>
<td>Psychiatric Illness</td>
<td>17 (8.5%)</td>
</tr>
<tr>
<td>Dementia</td>
<td>6 (3%)</td>
</tr>
<tr>
<td>Encephalopathy</td>
<td>2 (1%)</td>
</tr>
<tr>
<td>Seizures</td>
<td>2 (1%)</td>
</tr>
<tr>
<td>Spastic paraparesis</td>
<td>1 (0.5%)</td>
</tr>
</tbody>
</table>

### Table 3. Relations Between Cardiovascular Risk Factors and Clinical Features

<table>
<thead>
<tr>
<th></th>
<th>Migraine</th>
<th>Encephalopathy</th>
<th>Depression</th>
<th>Stroke</th>
<th>Dementia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>0.95 (0.92–0.98)*</td>
<td>1.00 (0.96–1.04)</td>
<td>1.01 (0.99–1.04)</td>
<td>1.05 (1.03–1.08)*</td>
<td>1.08 (1.04–1.13)*</td>
</tr>
<tr>
<td>Male sex</td>
<td>0.62 (0.32–1.17)</td>
<td>1.23 (0.50–3.05)</td>
<td>0.57 (0.31–0.84)</td>
<td>1.29 (0.74–2.27)</td>
<td>2.51 (1.18–5.39)†</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.69 (0.33–1.44)</td>
<td>1.70 (0.64–4.50)</td>
<td>0.81 (0.40–1.63)</td>
<td>2.57 (1.29–5.14)*</td>
<td>0.80 (0.32–1.97)</td>
</tr>
<tr>
<td>Ever-smoker</td>
<td>0.75 (0.39–1.42)</td>
<td>0.57 (0.23–1.44)</td>
<td>1.81 (1.00–3.26)</td>
<td>1.13 (0.65–1.96)</td>
<td>0.85 (0.41–1.77)</td>
</tr>
<tr>
<td>Smoking, pack-years</td>
<td>0.98 (0.95–1.01)</td>
<td>0.98 (0.92–1.04)</td>
<td>0.99 (0.96–1.02)</td>
<td>1.07 (1.03–1.11)*</td>
<td>1.02 (0.99–1.06)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>0.55 (0.25–1.20)</td>
<td>0.645 (0.245–1.697)</td>
<td>1.35 (0.70–2.62)</td>
<td>1.06 (0.57–1.96)</td>
<td>1.61 (0.65–4.00)</td>
</tr>
<tr>
<td>Hyperhomocysteinemia</td>
<td>1.47 (0.46–4.70)</td>
<td>1.56 (0.39–6.18)</td>
<td>1.27 (0.49–3.11)</td>
<td>0.99 (0.40–2.43)</td>
<td>0.74 (0.16–3.53)</td>
</tr>
<tr>
<td>Body mass index $&gt;25$ kg/m$^2$</td>
<td>0.61 (0.26–1.46)</td>
<td>0.64 (0.23–1.78)</td>
<td>1.52 (0.73–3.18)</td>
<td>1.39 (0.70–2.75)</td>
<td>1.12 (0.39–3.23)</td>
</tr>
</tbody>
</table>

Values shown are ORs with 95% CIs.

* $P < 0.01$, † $P < 0.02$. 

Figure 1. Distribution of age at onset of different symptoms of CADASIL.
enience of nor the age at onset of encephalopathy was associated with cardiovascular risk factors.

Psychiatric Illnesses
Seventy-five (37.5%) individuals had experienced a psychiatric illness requiring treatment. This included 70 (35%) with a depressive disorder and 1 with each of the following conditions: bipolar disorder, self-harm/parasuicide, schizophrenia, alcohol dependence, and acute psychosis. Mean age at onset of depressive illness was 41 (SD 10.2) years, and the distribution of the age at onset is shown in Figure 1. There were no associations between risk factors, or a history of stroke, and depression.

Dementia
Thirty-four (16%) individuals had dementia, and the mean age at diagnosis was 54.6 (SD 9.7) years. Male sex was associated with an increased risk of dementia (OR = 2.51; 95% CI, 1.18 to 5.36; P = 0.017), and this remained after covarying for age and cardiovascular risk factors (OR = 2.52; 95% CI, 1.05 to 6.05; P = 0.039) but not when after adjusting for a history of stroke. A history of stroke was associated with an increased risk of dementia after covarying for age, sex, and risk factors (OR = 6.08; 95% CI, 2.07 to 17.91; P = 0.001). There were no other associations between risk factors and dementia.

Disability
The modified Rankin score was 0 in 71 (35.5%), 1 in 54 (27%), 2 in 37 (18.5%), 3 in 21 (10.5%), 4 in 12 (6%), and 5 in 5 (2.5%). Rankin score was associated with a history of stroke, even after adjusting for age and risk factors (OR = 5.43; 95% CI, 2.03 to 14.52; P = 0.001). There were no other independent associations between risk factors or clinical outcomes and functional disability.

Genotypic Spectrum
The distribution of NOTCH3 mutations is shown in supplemental Table I, available online at http://stroke.ahajournals.org. All mutations identified were missense mutations involving a gain or loss of a cysteine residue. The majority of mutations (71.4%) were in exon 4, as previously described. Four previously unreported mutations were found: T697C in exon 5, C1279T and G1370C in exon 8, and C1774T in exon 11. There was no association between mutation site defined by codon or exon and the presence or age at onset of clinical phenotypes. There was no evidence of significant intrafamilial clustering of clinical phenotypes.

Discussion
This study describes the clinical spectrum of CADASIL in a large cohort of prospectively recruited patients from a single country and explores the factors that modulate the clinical phenotype. It confirms previous data showing no relation between genotype and clinical phenotype, but it does suggest that cardiovascular risk factors may modulate the disease and increase disease severity.

The clinical features of our cohort were similar to those in smaller cohorts previously described. Migraine was the most prevalent clinical feature, affecting 75% of individuals, and the most frequent first feature. Stroke was a presenting feature in fewer than one fifth of the cases, although the prevalence of stroke was 50% in our cohort, with a mean age at onset of 46 years.

Our data demonstrate a number of characteristic features of migraine in CADASIL. Ninety percent of migraineurs experienced migraine with aura with a wide variety of aura types, frequently in >1 domain. Prolonged aura, lasting >1 hour, was present in one third of individuals, and 16% reported acute confusion associated with migraine. Our data also confirm the frequency of reversible encephalopathy, or “CADASIL coma,” which was present in 10% of individuals and was the first major event in 9%. In terms of clinical presentation, this has some similarities to migraine coma seen in familial hemiplegic migraine, and in all but 1 of the cases in our series, there was a history of the attack after a typical migraine with aura. The underlying mechanism remains uncertain, but it is possible that it represents part of the CADASIL migraine spectrum. This is perhaps supported by some cases in which individuals experienced migraine attacks with prolonged confusion as well as encephalopathic episodes.

Centralized review of brain imaging demonstrated that, in almost all cases, the pattern of infarction was lacunar, as would be expected in small-vessel disease. There was 1 cortical infarct that could have represented an incidental infarct. In addition, 1 individual experienced a brainstem hemorrhage. This individual was on warfarin therapy and adds to a number of case reports demonstrating the risk of hemorrhage in CADASIL patients. Gradient-echo MRI has shown a high prevalence of microbleeds in CADASIL, which may predict intracerebral hemorrhage, and emphasizes that caution should be used when prescribing anticoagulation therapy to CADASIL patients.

Genetic analysis revealed a large number of mutations, but all were stereotyped missense mutations resulting in a gain or loss of a cysteine residue within epidermal growth factor–like domains. There was no relation between mutation site and any clinical feature. This lack of association, combined with the marked phenotypic variation within families with the
same mutation, implicates other factors in the modulation of clinical phenotype. A recent study found a high intrafamilial heritability for white-matter lesion volume, suggesting additional, and as yet unidentified, modulating genes.$^5$

Our data also suggest that cardiovascular risk factors may modulate the phenotype. This study is the first to report an increased stroke risk in the presence of hypertension in CADASIL. Hypertension is a well-established risk factor for sporadic small-vessel disease. Support for the association in CADASIL is provided by MRI studies, in which higher blood pressure was associated with brain volume loss and increased T2 lesion load on serial imaging.$^7$ Smoking was associated with an earlier onset of stroke. Smoking may therefore predispose to acute occlusion of perforating arteries in CADASIL. However, confirming that cardiovascular risk factors do modulate severity in CADASIL will require longitudinal studies to prove causality. Nevertheless, the associations with hypertension and smoking suggest that, until such data are obtained, cardiovascular risk factors should be treated aggressively in patients with CADASIL.

We found a relatively low prevalence of dementia (16%) in this study, with a mean age at onset of 9 years later than that of stroke. This may partly reflect the fact that we studied a younger cohort. It also reflects the fact that definitions of dementia rely on the involvement of memory and language domains typically affected in cortical dementias, which may be absent, despite marked cognitive impairment in “subcortical dementias.”$^2$ We found that stroke was a risk factor for dementia, and in addition, that male sex was associated with increased dementia. One large retrospective study has suggested that male sex may be associated with poor prognosis in CADASIL, with earlier immobility and death.$^2$ However, this study did not explore potential sex differences in risk factors. There are no reported sex differences in brain damage, as assessed by white-matter lesion volume.$^7$

We found that a history of migraine aura was associated with a lower stroke risk. Whether this represents a real association is uncertain. It could represent recall bias, with stroke cases being older and having cognitive difficulties leading to reduced recall of migraine history. Some authors have reported a tendency for migraine remission after the first stroke cases being older and having cognitive difficulties leading to reduced recall of migraine history. Some authors have reported a tendency for migraine remission after the first stroke. This may party reflect the fact that we studied a younger cohort. It also reflects the fact that definitions of dementia rely on the involvement of memory and language domains typically affected in cortical dementias, which may be absent, despite marked cognitive impairment in “subcortical dementias.”$^2$ We found that stroke was a risk factor for dementia, and in addition, that male sex was associated with increased dementia. One large retrospective study has suggested that male sex may be associated with poor prognosis in CADASIL, with earlier immobility and death.$^2$ However, this study did not explore potential sex differences in risk factors. There are no reported sex differences in brain damage, as assessed by white-matter lesion volume.$^7$

We found that a history of migraine aura was associated with a lower stroke risk. Whether this represents a real association is uncertain. It could represent recall bias, with stroke cases being older and having cognitive difficulties leading to reduced recall of migraine history. Some authors have reported a tendency for migraine remission after the first stroke. This may party reflect the fact that we studied a younger cohort. It also reflects the fact that definitions of dementia rely on the involvement of memory and language domains typically affected in cortical dementias, which may be absent, despite marked cognitive impairment in “subcortical dementias.”$^2$ We found that stroke was a risk factor for dementia, and in addition, that male sex was associated with increased dementia. One large retrospective study has suggested that male sex may be associated with poor prognosis in CADASIL, with earlier immobility and death.$^2$ However, this study did not explore potential sex differences in risk factors. There are no reported sex differences in brain damage, as assessed by white-matter lesion volume.$^7$

In summary, in this large, prospectively recruited, CADASIL population from a single country, we found no genotype-phenotype correlations. In contrast, 2 conventional cardiovascular risk factors, smoking and hypertension, were associated with age of stroke onset. This emphasizes the need for careful risk factor control in CADASIL patients.

Disclosures

None.

References


Clinical Spectrum of CADASIL and the Effect of Cardiovascular Risk Factors on Phenotype: Study in 200 Consecutively Recruited Individuals
Ponieh Adib-Samii, Glen Brice, Roswell J. Martin and Hugh S. Markus

Stroke. 2010;41:630-634; originally published online February 18, 2010;
doi: 10.1161/STROKEAHA.109.568402

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/41/4/630