Right-to-Left Shunt in CADASIL Patients
Prevalence and Correlation With Clinical and MRI Findings

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Background and Purpose—A high prevalence of right-to-left shunt (RLS) was described in a family of patients with CADASIL, a rare cerebral arteriopathy attributable to Notch3 gene mutations. The aim of this study was to determine the prevalence of RLS in patients with CADASIL and possible relation to clinical phenotype and cerebral MRI lesion load.

Methods—Twenty-three CADASIL patients underwent Transcranial Doppler with gaseous contrast to assess RLS. Correlations between RLS, clinical features, and MRI lesion volume (LV) were determined.

Results—Large RLS was diagnosed in 47% of patients. No significant clinical or MRI differences were found between patients with and without RLS.

Conclusion—We found a high prevalence of RLS in our group of CADASIL patients. This may not be a coincidence, but can be rather related to the role of the Notch receptor family in the development of cardiovascular system. (Stroke. 2008; 39:2155-2157.)

Key Words: right-to-left shunt • patent foramen ovale • Transcranial Doppler • CADASIL.

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a hereditary small vessels disease determined by mutations in the Notch3 gene.1 Main clinical manifestations include recurrent strokes, dementia, and migraine with aura (MA).2 The reasons for the extreme variability in presentation and clinical progression are still not understood. Recently, right-to-left shunt (RLS) attributable to patent foramen ovale (PFO) was detected by Transcranial Doppler (TCD) with gaseous contrast in a family with CADASIL presenting with MA.3

The aim of the present study was to establish the prevalence of RLS in a larger population of CADASIL patients and to investigate a possible correlation between RLS, clinical picture, and cerebral MRI lesion load.

Materials and Methods

Patients
Twenty-three consecutive CADASIL patients with positive genetic test were recruited for the study. Demographic and clinical data, including age, sex, presence of TIA/stroke, MA, cognitive impairment and behavioral dysfunction and the concomitant history of hypertension, smoking, hypercholesterolaemia, diabetes mellitus, and hyperhomocystinemia were recorded. The local medical ethics committee approved the study. Informed consent was obtained from all participants.

Magnetic Resonance Imaging
Brain MRI was acquired in 22/23 CADASIL patients to evaluate the white matter (WM) lesion volume (LV). All patients were examined using the same MR protocol, using a Philips Gyroscan operating at 1.5 T (Philips Medical Systems). Dual-echo, turbo spin-echo (TR/TE1/TE2 = 2075/30/90 ms, 50 contiguous 3-mm slices) images, yielding proton density–weighted and T2-weighted (T2-W) images, and T1-weighted (T1-W) gradient echo images (TR/TE = 35/10 milliseconds, 256×256 matrix, 50 contiguous 3-mm slices) were acquired in the transverse plane. Classification of T2-W and T1-W LV was performed in each patient by a single observer, unaware of subject identity, using a segmentation technique based on user supervised local thresholding.4 In T2-W and T1-W images, total LV was calculated by multiplying lesion area by slice thickness.

Transcranial Doppler
Conventional TCD was performed by an examiner unaware of the diagnosis, to evaluate the ultrasound permeability of the temporal acoustic walls and to exclude stenosis of intracranial vessels (Sonos 5500, Philips, probe 1.8–3.6 MHz). TCD with bilateral monitoring (DWL Multidop X4, DWL) was used to assess RLS while an agitated solution of 9 mL of saline mixed with 1 mL air was injected into an antecubital vein during normal ventilation and Valsalva maneuver. According to established criteria,5 RLS was diagnosed when at least one Microembolic Signal (MES) appeared in the Doppler spectrum within 40 seconds after the beginning of the procedure. Patients were divided into RLS positive and negative groups. Microbubbles (MB) number was used to assess the severity of the shunt in a 4-level categorization: (1) 0 MB (negative result); (2) 1 to 10 MB; (3) >10 MB and no curtain, and (4) curtain.

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2155
Table 1. Main Clinical and Demographic Characteristics (Overall CADASIL Population; n=23)

<table>
<thead>
<tr>
<th>Main Characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y, mean±SD (range)</td>
<td>49±11 (27 to 73)</td>
</tr>
<tr>
<td>Sex</td>
<td>15 males</td>
</tr>
<tr>
<td></td>
<td>8 females</td>
</tr>
<tr>
<td>Arterial hypertension (95% CI)</td>
<td>7/23 30% (16 to 51)</td>
</tr>
<tr>
<td>Hyperhomocystinemia (95% CI)</td>
<td>4/23 17% (7 to 37)</td>
</tr>
<tr>
<td>Past or current smoker (95% CI)</td>
<td>6/23 26% (13 to 46)</td>
</tr>
<tr>
<td>Asymptomatic patients (95% CI)</td>
<td>4/23 17% (7 to 37)</td>
</tr>
<tr>
<td>TIA/stroke (95% CI)</td>
<td>15/23 65% (45 to 81)</td>
</tr>
<tr>
<td>Migraine (95% CI)</td>
<td>7/23 30% (16 to 51)</td>
</tr>
<tr>
<td>Cognitive impairment (95% CI)</td>
<td>9/23 39% (22 to 59)</td>
</tr>
<tr>
<td>Behavioural dysfunction (95% CI)</td>
<td>14/23 61% (41 to 78)</td>
</tr>
</tbody>
</table>

Statistical Analysis
Data are expressed as mean and standard deviation (SD). The Mann Whitney Wilcoxon test has been used to compare the quantitative variables between RLS positive and RLS negative patients. For the nonquantitative variables the odd ratios has been used, and the significance has been based on the normal approximation. A probability value lower than 0.05 has been considered statistically significant.

Results
The main clinical characteristics of the study population are summarized in Table 1.

Fifteen patients had experienced at least 1 cerebrovascular event (65%). Migraine was recorded in 7 patients (30%). Brain MRI examination performed in 22/23 CADASIL patients showed a T2-W LV of 55.1 ± 50.8 cm³ and a T1-W LV of 27.2 ± 29.3 cm³. All CADASIL patients had MRI WM lesions.

TCD assessment of RLS was performed in 21 patients. From 2 of 23 there was no acoustic window. RLS was diagnosed in 15 out of 21 patients (71%). Overall, patients with and without RLS showed overlapping demographic, clinical, and MRI characteristics (Table 2). Eight patients had level (3) and 2 level (4) of RLS (47%). No significant differences on clinical phenotype or the amount of MRI changes were found between RLS-positive and RLS-negative patients (Table 2).

Discussion
Our results are the first evidence of a high prevalence of large RLS in relatively large group of CADASIL patients. This is twice more than what we found in cerebrovascular non-CADASIL patients in our laboratory (data not shown). This finding is probably attributable to PFO, a hemodynamically silent interatrial communication reported to occur in about 25% of the general population. This cardiac septal defect is associated with several pathological conditions such as “cryptogenic” ischemic stroke, usually occurring in subjects under 55 years of age. The prevalence of PFO with large RLS in stroke ranges from 40% to 56%. In addition, a high prevalence of RLS (41%) and PFO (48%) has been recorded in patients with MA and in some families common dominant inheritance has been proposed for PFO and MA.7

Ischemic strokes and MA are the most common clinical manifestations of CADASIL, a dominantly inherited mono- genic disease attributable to Notch3 gene mutations.

In our study, the high prevalence of RLS in CADASIL patients might not be a coincidence but may rather suggest a common genetic origin of CADASIL and the cardiac septal defect. Indeed, Notch signaling regulates cell differentiation during cardiovascular system development.8 In adults, Notch3 is expressed exclusively in vascular smooth muscle cells (VSMCs). Gradual degeneration of VSMCs leads to progressive wall thickening and luminal narrowing in small penetrating arteries. Reduced cerebral blood flow finally causes lacunar infarcts and leukoencephalopathy leading to motor deficits and subcortical vascular dementia.8 Moreover, Notch3 mutations may have a role in abnormal development of the endocardial cushion, as suggested by experimental work showing that Notch3 is also expressed in heart precursors during embryogenesis and that the Notch pathway is

Table 2. Descriptive Statistics of Clinical Characteristics and Volume of T1-W and T2-W Lesions in Patients (n=21) With and Without RLS

<table>
<thead>
<tr>
<th>Demographic characteristics</th>
<th>RLS-Positive Patients, n=15 (71%)</th>
<th>RLS-Negative Patients, n=6 (29%)</th>
<th>Odds Ratio (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y, mean±SD</td>
<td>45±10</td>
<td>54±11</td>
<td>...</td>
<td>0.17</td>
</tr>
<tr>
<td>Sex</td>
<td>12 males</td>
<td>3 males</td>
<td>...</td>
<td>0.16</td>
</tr>
<tr>
<td>Disease duration, mean±SD</td>
<td>5±5</td>
<td>6±7</td>
<td>...</td>
<td>0.99</td>
</tr>
<tr>
<td>Clinical characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIA/stroke (95% CI)</td>
<td>10 (67%) (42 to 85)</td>
<td>4 (67%) (30 to 90)</td>
<td>1 (0.134 to 7.4514)</td>
<td>0.500</td>
</tr>
<tr>
<td>Migraine (95% CI)</td>
<td>4 (27%) (11 to 52)</td>
<td>2 (33%) (10 to 70)</td>
<td>0.75 (0.097 to 5.817)</td>
<td>0.613</td>
</tr>
<tr>
<td>Cognitive impairment (95% CI)</td>
<td>6 (40%) (20 to 64)</td>
<td>2 (33%) (10 to 70)</td>
<td>1.35 (0.186 to 9.872)</td>
<td>0.381</td>
</tr>
<tr>
<td>Behavioral dysfunction (95% CI)</td>
<td>9 (60%) (36 to 80)</td>
<td>4 (67%) (30 to 90)</td>
<td>0.74 (0.101 to 5.389)</td>
<td>0.619</td>
</tr>
<tr>
<td>T1-W LV, mean±SD</td>
<td>26.3±29.3</td>
<td>25.4±30.5</td>
<td>...</td>
<td>0.59</td>
</tr>
<tr>
<td>T2-W LV, mean±SD</td>
<td>56.9±55.8</td>
<td>45.4±50.1</td>
<td>...</td>
<td>0.54</td>
</tr>
</tbody>
</table>

Values are mean±SD or n (%).
crucial role in regulating atrioventricular morphogenesis, including cardiac valves and septa.\textsuperscript{8}

The possible influence of other genetic or environmental factors in the occurrence of RLS cannot be ruled out and should be evaluated by studying nonmutation carriers of the same families.

Despite the high incidence of RLS in our CADASIL population, this hemodynamic defect was not correlated with clinical severity or MRI lesion load. Many factors, which probably include subjective (genetic) variability in response to injury as well as the ability to activate mechanisms of adaptation to compensate damage, concur in the full clinical picture of this complex disease. Larger studies are probably necessary to provide definite insights into the prevalence and role of cardiac shunts in modulating clinical phenotype in CADASIL.

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Disclosures
None.

References
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