Arterioles of the Lenticular Nucleus in CADASIL

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Background and Purpose—In cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) the arteriopathy leads to recurrent infarcts in cerebral white matter (WM) and deep gray matter (GM), whereas cortex is spared. To assess the pathogenesis of deep GM infarcts, we analyzed structural changes in arterioles of the lenticular nucleus (LN) in 6 CADASIL patients.

Methods—Five elderly and one 32-year-old deceased CADASIL patients were studied. Seven elderly and 4 young deceased persons without cerebrovascular diseases served as controls. In addition to immunohistochemical analysis the external and luminal diameters of arterioles in the LN, cerebral cortex and WM were measured. The thickness of arteriolar wall and sclerotic index were calculated.

Results—In CADASIL patients, LN arterioles were immunoreactive for the extracellular domain of Notch3 and collagen I, whereas α-smooth muscle actin staining was irregular or negative. No major leakage of plasma fibrinogen or fibronectin was observed. Although in patients the walls of LN arterioles were significantly thicker than in controls, definite stenosis was not observed. Arteriolar lumina in the LN were not only significantly larger than in the WM, where most lacunar infarcts in CADASIL occur, but also larger than in cortical GM, where infarcts virtually never exist.

Conclusions—Fibrotic thickening of the arteriolar walls without consequent stenosis occurs in the LN of CADASIL patients. The pathogenesis of lacunar infarcts in the WM and LN seem to be different, stenosis in the former and probably hemodynamic disturbances in the latter. (Stroke. 2006;37:2242-2247.)

Key Words: CADASIL ■ cerebral arteries ■ fibrosis ■ lenticular nucleus ■ stenosis

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a hereditary small arteriolar disease that is caused by over 130 different mutations in the NOTCH3 gene1,2 (Myykkänen et al, unpublished data, 2006). The pathological hallmark of CADASIL is degeneration of vascular smooth muscle cells (VSMC) associated with deposition of the extracellular domain of the Notch3 protein (N3ECD) and granular osmiophilic material on the degenerating VSMC in arteries of most organs.3 The vascular changes cause ischemic brain lesions, which gradually lead to cognitive decline and eventually to dementia.4

We have previously demonstrated stenosis of cerebral white matter (WM) arterioles with fibrosis and thickening of their walls attributable to accumulation of various extracellular matrix components which appear to finally lead to decreased cerebral blood flow (CBF) of such a severity that lacunar infarcts as well as diffuse changes in the WM ensue.5,6 We also showed that arterioles in the cerebral cortex were markedly less severely affected, which is one explanation for the absence of infarcts in cerebral cortical gray matter (GM).5,6 Remarkably, multiple small lacunar infarcts are also found in the deep GM of basal ganglia. The reason for the different vulnerability of the cortical and deep GM has not been addressed, but may depend on differential anatomy of arterioles in these 2 vascular beds.

In this article we present an analysis of arteriolar pathology, including the external diameter of arterioles, diameter of arteriolar lumen, thickness of arteriolar wall and sclerotic index (SI) in the lenticular nucleus (LN; ie, putamen and globus pallidus) in 5 deceased elderly and one 32-year-old deceased CADASIL patient.

Materials and Methods

Patients and Controls

Five deceased elderly CADASIL patients (2 females and 3 males, mean age 63.4±2.9 years, range 60 to 68 years, from 4 different Finnish

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families, with molecular genetically confirmed C475T (R133C) NOTCH3 mutation and one 32-year-old male deceased patient, unexpectedly died of massive middle cerebral artery infarct, from a Swedish family with T98C (C174R) mutation were available for this study. Seven deceased elderly (3 females and 4 males, mean age 64.3 ± 7.1 years, range 56 to 73 years) and 4 deceased young persons (2 females and 2 males, mean age 29.3 ± 2.5, range 26 to 32) without known cerebrovascular diseases served as LN controls. Four deceased elderly and 3 deceased young persons (1 female and 2 males, mean age 28.3 ± 2.1, range 26 to 30) with known cerebrovascular diseases served as lobar controls.

**Histology and Immunohistochemistry**

Samples from routinely fixed brains were embedded in paraffin for light microscopy. Sections covering the LN, frontal cortical GM and underlying WM were cut and stained with hematoxylin and eosin to be used in the morphometric study. Additional sections were immunostained for detection of collagen I (polyclonal rabbit antibody; Biotrend, Cologne, Germany), α-smooth muscle actin (α-SMA; monoclonal mouse antibody; Sigma; St. Louis, USA), N3ECD (monoclonal mouse antibody, a kind gift from Dr A. Joutel), fibrinogen and fibronectin. The bound primary antibodies were visualized by using appropriate peroxidase-labeled secondary antibodies (Vector Laboratories) with diaminobenzidine as chromogen and hematoxylin as counterstain.

**Morphometric Study**

The morphometric analysis was performed as previously described. Briefly, all transversely cut arterioles in the LN, frontal cortical GM and underlying WM with internal diameter ≤ 50 μm in a section were examined (selection based on our previous results, and on the fact that majority of arterioles have luminal diameters of 10 to 45 μm or below 70 μm²) and arterioles of this size are decisive for the small vessel ischemia). The internal and external diameters of arterioles were measured using tailor-made software for on-screen measurements (Pit Oy). The external diameter was measured as the diameter spanning external boundaries of the adventitial connective tissue. The thickness of the arteriolar wall was defined as (external diameter−internal diameter)/2. The SI was calculated as 1−(internal diameter/external diameter).

**Statistical Analysis**

Data are presented as means ± SD. The external diameter of arterioles, diameter of arteriolar lumen, thickness of arteriolar wall and SI in the LN of the elderly and young CADASIL patients were compared with those of the controls using one-way ANOVA. In CADASIL patients we also compared the values above in LN versus cortical GM and WM. Values of P < 0.01 were considered significant.

**Results**

**Histology and Immunohistochemistry**

In the LN arterioles of the elderly CADASIL patients basophilic granular material accumulated in the tunica media (Figure 1A), where α-SMA staining for VSMC was irregular or negative (Figure 1I). Adventitia immunostained strongly for collagen I (Figure 1J). Clear immunopositivity for N3ECD existed in the degenerating tunica media (Figure 1K). Immunoreactivity for fibrinogen or fibronectin, as a marker of blood–brain barrier (BBB) leakage, was very uncommon around arterioles with degenerating tunica media (Figure 1K). Immunoreactivity for fibrinogen or fibronectin, as a marker of blood–brain barrier (BBB) leakage, was very uncommon around arterioles with degenerating tunica media (Figure 1K).

**Morphometric Analysis of the LN Arterioles**

The 4 indicators of arteriolar fibrosis and stenosis, external diameter of arterioles, diameter of arteriolar lumen, thickness of arteriolar wall and SI, as well as the numbers of arterioles measured in the LN, cortical GM and WM are given in the Table.

**External Diameter of Arterioles**

The external diameter of LN arterioles in the elderly CADASIL patients was almost significantly greater than in the respective controls (P = 0.012), whereas in the young CADASIL patient there was only a similar trend (P = 0.14; Figure 2A).

**Diameter of Arteriolar Lumen**

Diameter measurements of the arteriolar lumen indicated that there was only relatively mild stenosis, which was markedly less severe than in WM arterioles. The diameter of the LN arteriolar lumen in both the elderly and young CADASIL patients was somewhat smaller than those in the elderly and young controls, but the differences did not reach significance (P = 0.02, P = 0.04, respectively; Figure 2B).

**Thickness of Arteriolar Wall**

Analysis of thickness of the LN arteriolar wall revealed that CADASIL also causes remarkable fibrosis and thickening of the arteriolar walls in the LN though less severe than in the WM. In both the elderly and young CADASIL patients the thickness of the arteriolar wall was significantly greater than those in the corresponding controls (P < 0.01 for both age groups; Figure 2C).

**Sclerotic Index**

The SI of LN arterioles in both the elderly and young CADASIL patients was significantly greater than that of the respective controls (P < 0.01, P < 0.01, respectively; Figure 2D).

**Comparison of the LN Arterioles With Cortical GM or WM Arterioles**

In the elderly CADASIL patients the LN arterioles were more severely affected than those in cortical GM, except for the diameter of the arteriolar lumen, which was greater in LN than in cortical GM arterioles (Table; Figure 2A through 2D). All these differences were statistically significant (P < 0.01) and they indicated more severe fibrosis but larger arterioles (no stenosis) in LN than in cortical GM.

All 4 indicators of arteriolar fibrosis and stenosis demonstrated that the LN arterioles in the elderly CADASIL patients were less severely affected than those in the WM (Table; Figure 2A through 2D). All these differences were statistically significant (P < 0.01).

In the young CADASIL patient the differences between LN versus cortical GM or WM showed similar trends, but the differences between the indicator values reached statistical significance (P < 0.01) only for the external diameter of arterioles in LN versus cortical GM and for SI in LN versus WM (Table; Figure 2A through 2D).

**Comparison of the Elderly and Young CADASIL Patients**

There was no significant difference in the external diameter of LN arterioles (P = 0.68; Figure 2A), diameter of LN arteriolar lumen (P = 0.28; Figure 2B), thickness of LN arteriolar wall
In both the elderly and young CADASIL patients the LN arteriolar walls were significantly thicker than in the controls. However, the diameter of LN arteriolar lumen was approximately similar to that of controls. Moreover, in the elderly CADASIL patients the diameter of the arteriolar lumen in the LN was significantly larger than in the WM, where the majority of lacunar infarcts in CADASIL occur. Also, the diameter of the arteriolar lumen in the LN was even larger than in cortical GM, where infarcts hardly ever occur. Thus, unlike the situation seen in WM arterioles in CADASIL, the thickening of the arteriolar walls in the LN was not associated with significant stenosis. This was also reflected in the lower SI values of LN as compared with WM arterioles. This suggests that CBF is probably not signifi-
significantly reduced in the LN. Indeed, in our positron emission tomographic study on young CADASIL patients (mean age 32.8 years) we found that CBF in the putamen was actually greater than that in the cerebral cortex and even slightly higher in patients than in the controls.11 Similarly, Chabriat et al reported no difference in the CBF of basal ganglia between the patients and controls.12 However, in our other positron emission tomographic study on 2 older CADASIL patients we found in the putamen a moderately (but clearly less than in the WM) reduced CBF in one homozygous (52-year-old) and one heterozygous (53-year-old) patient. However, the unavoidably lower age of the controls (41.4 years) may have influenced this result.13 Similarly, the markedly older age of CADASIL patients as compared with controls (43 versus 27 years) in a single photon emission computed tomographic study could explain the reduced CBF in the basal ganglia.14 Thus, the basic flow values alone probably do not explain the appearance of lacunar infarcts in LN.

Because the LN represents GM, though deep subcortical GM, these results are consistent with our previous studies in (53-year-old) patient. However, the unavoidably lower age of the controls (41.4 years) may have influenced this result.13 Similarly, the markedly older age of CADASIL patients as compared with controls (43 versus 27 years) in a single photon emission computed tomographic study could explain the reduced CBF in the basal ganglia.14 Thus, the basic flow values alone probably do not explain the appearance of lacunar infarcts in LN.

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### Table: External Diameter of Arterioles, Diameter of Arteriolar Lumen, Thickness of Arteriolar Wall and Sclerotic Index in Lenticular Nucleus, Lobar Grey and White Matter in Elderly, Young CADASIL Patients and Controls

<table>
<thead>
<tr>
<th></th>
<th>External Diameter of Arterioles</th>
<th>Diameter of Arteriolar Lumen</th>
<th>Thickness of Arteriolar Wall</th>
<th>Sclerotic Index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LN</td>
<td>GM</td>
<td>WM</td>
<td>LN</td>
</tr>
<tr>
<td>Elderly CADASIL</td>
<td>50.33±22.30</td>
<td>38.52±10.14*</td>
<td>55.78±23.35†</td>
<td>19.82±9.25</td>
</tr>
<tr>
<td>(N=5)</td>
<td>(n=486)</td>
<td>(n=171)</td>
<td>(n=1056)</td>
<td>(n=486)</td>
</tr>
<tr>
<td>Elderly LN controls</td>
<td>46.85±15.88</td>
<td>21.49±10.08</td>
<td></td>
<td></td>
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<tr>
<td>(N=7)</td>
<td>(n=309)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elderly lobar controls</td>
<td>36.97±8.01</td>
<td>43.41±10.78</td>
<td>19.58±7.43</td>
<td>28.29±7.45</td>
</tr>
<tr>
<td>(N=4)</td>
<td>(n=75)</td>
<td>(n=69)</td>
<td>(n=49)</td>
<td>(n=49)</td>
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<tr>
<td>Young CADASIL</td>
<td>48.96±15.39</td>
<td>37.77±8.59*</td>
<td>49.93±20.30</td>
<td>21.61±10.25</td>
</tr>
<tr>
<td>(N=1)</td>
<td>(n=35)</td>
<td>(n=69)</td>
<td>(n=61)</td>
<td>(n=35)</td>
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<tr>
<td>Young LN controls</td>
<td>43.61±10.69</td>
<td>25.34±9.02</td>
<td></td>
<td></td>
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<tr>
<td>(N=4)</td>
<td>(n=134)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young lobar controls</td>
<td>37.00±7.13</td>
<td>44.36±11.33</td>
<td>22.12±6.47</td>
<td>26.09±8.59</td>
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<tr>
<td>(N=3)</td>
<td>(n=82)</td>
<td>(n=52)</td>
<td>(n=82)</td>
<td>(n=52)</td>
</tr>
</tbody>
</table>

N indicates No. of patients analyzed; n: No. of arterioles measured.
One-way ANOVA, \( P<0.01 \).
*LN vs GM.
†LN vs WM.
‡CADASIL LN vs LN controls.
§Elderly vs young CADASIL patients.

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Figure 2. A, The external diameter of arterioles; B, diameter of arteriolar lumen; C, thickness of arteriolar wall; and D, SI of the LN, GM and WM in the elderly, young CADASIL patients and LN controls.
Continued

<table>
<thead>
<tr>
<th>Thickness of Arteriolar Wall</th>
<th>LN</th>
<th>GM</th>
<th>WM</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.26±8.79</td>
<td>10.60±3.82*</td>
<td>20.49±8.84†</td>
<td>0.60±0.13</td>
</tr>
<tr>
<td>(n=486)</td>
<td>(n=171)</td>
<td>(n=1056)</td>
<td>(n=486)</td>
</tr>
<tr>
<td>12.68±5.02‡</td>
<td></td>
<td>0.55±0.12‡</td>
<td>0.48±0.12</td>
</tr>
<tr>
<td>(n=309)</td>
<td></td>
<td>(n=309)</td>
<td>(n=75)</td>
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<tr>
<td>13.67±4.09</td>
<td>10.28±2.43</td>
<td>15.73±6.19</td>
<td>0.57±0.10</td>
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<tr>
<td>(n=35)</td>
<td>(n=69)</td>
<td>(n=61)</td>
<td>(n=35)</td>
</tr>
<tr>
<td>9.13±2.47‡</td>
<td>7.44±1.59</td>
<td>9.13±2.95</td>
<td>0.43±0.10‡</td>
</tr>
<tr>
<td>(n=134)</td>
<td>(n=82)</td>
<td>(n=52)</td>
<td>(n=134)</td>
</tr>
</tbody>
</table>

which we demonstrated that the thickening and narrowing of the arterioles in the GM of both cerebral cortex and retina are less severe than in the WM.\(^5,6,15\) Considering the larger arteriolar lumen in the LN than in cortical GM, it is paradoxical that multiple lacunar infarcts occur in the LN but not in cortical GM. This implies that in CADASIL the pathogenesis of lacunar infarcts in the LN (deep GM), is different from that in the cerebral WM, where definite stenosis, even occlusions, were verified in our postmortem analysis.\(^5,6\) Indeed, Uehara et al reported that the risk factors and thus most likely causes of silent cerebral infaracts in WM and basal ganglia were different.\(^16\) Lacunar infarcts in WM are considered to be most commonly attributable to intrinsic small vessel disease in the penetrating arteries, a situation prevailing in CADASIL, whereas the lesions in basal ganglia appear to be more often associated with atherosclerosis, emboli and carotid artery stenosis.\(^16,17\) However, the studies on lacunar infarcts have all too infrequently distinguished between the WM and deep GM lesions.

At present there is no definite evidence for thrombotic or thromboembolic etiology of lacunar infarcts in CADASIL patients. Stenosis in the main cerebral arteries has recently been reported in 5 of 13 (38%) elderly CADASIL patients\(^18\) although such stenoses are common in this age group and may well be coincidental. Whether stenosis in the main cerebral arteries has an important role in the development of lacunar infarcts has been a controversial issue.\(^19\) More often such stenoses would however preferentially cause larger infarcts, such as we observed in the 32-year-old CADASIL patient studied in this article.\(^6\)

The mechanism of lacunar infarcts that occur in association with large cerebral artery stenoses has been proposed to be hemodynamic.\(^20\) We suggest that in CADASIL, the major cause of lacunar infarcts in the LN is also hemodynamic. The reason for the higher CBF in the putamen of CADASIL patients as compared with controls is probably the loss of autoregulation attributable to the VSMC degeneration and fibrotic thickening and consequent rigidity of arterioles.\(^21\) This would result in high or normal CBF values, when systemic blood pressure is sufficient. However, when hypotensive episodes occur, the loss of contractility in the rigid fibrotic lenticulo-striate arteries allows the regional CBF to decrease to such a level that lacunar infarcts ensue. The reduced responses to vasodilator stimuli examined in vivo in a transgenic mouse model of CADASIL support this hypothesis.\(^22\) The higher metabolic demand for oxygen in the basal ganglia may further aggravate the ischemic insult.\(^23\)

Recently, a new hypothesis for the pathogenesis of lacunar infarctions was presented by Wardlaw.\(^17\) She proposed that the increased permeability of the BBB with chronic leakage of toxic substances through the walls of penetrating arterioles could be a decisive factor for lacunar stroke. The extensive T2-weighted MRI (T2w MRI) hyperintensities in CADASIL patients reflect edema, which was also verified by the increased diffusivity in diffusion tensor imaging.\(^24\) However, this edema is widely distributed and both diffuse and difficult to specifically associate with lacunar infarctions. Furthermore, although focal BBB breakdown does occur in CADASIL as shown by the presence of microbleeds in T2w MRI, these are most often located in cerebral cortex outside the T2w MRI hyperintensities of the WM and neither do they correlate with lacunar infarcts visible in T1w MRI.\(^25,26\) In concordance, our immunostainings did not disclose major leakage of large plasma proteins through the thickened, NSECD immunopositive walls of most penetrating arterioles in either cerebral WM or LN. Thus, the mechanism proposed by Wardlaw is an unlikely explanation for the lacunar infarcts in CADASIL.

In conclusion, our study demonstrates that in CADASIL patients fibrotic thickening of arteriolar walls occurs in LN, but without significant stenosis. We propose that the pathogenesis of the observed ischemia and lacunar infarcts in LN may probably be hemodynamic attributable to loss of autoregulation as a consequence of VSMC degeneration, fibrosis and rigidity of the arteriolar walls. Yet, small vessel thrombosis may also be responsible or contributory.

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Disclosures

None.

References


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