Cerebral Autosomal–Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) is the most common inherited cause of stroke and vascular dementia. In addition to prominent subcortical involvement, cortical dysfunction is suggested by the high frequency of migraine with aura, seizures, and reversible encephalopathy in CADASIL patients. Examination of the grey matter of CADASIL autopsies has demonstrated neuronal cell death by apoptosis. The symptoms and cortical pathology of CADASIL could be explained by stenosis and dysfunction of the leptomeningeal arteries, which are upstream of penetrating cortical vessels. But little dedicated attention has been paid to leptomeningeal arterial pathology in CADASIL. Several studies describe leptomeningeal arteries in general terms, with studies mostly focusing on single cases or patients who were not genetically characterized. The caliber of leptomeningeal arteries have not been studied in genetically proven CADASIL cohorts.

We analyzed the leptomeningeal arteries in North American patients with NOTCH3 mutations. Using a combination of standard and immunohistochemical staining and morphometry, we performed a quantitative analysis of leptomeningeal arterial substructure and caliber in CADASIL patients.

**Methods**

Frontal and temporal lobe sections were selected from autopsy samples from 3 groups: (1) CADASIL patients with cysteine-altering NOTCH3 mutations (n=6; age range, 46–83 years), (2) control subjects without symptoms of cerebrovascular disease (n=6; age range, 47–82 years), and (3) very old individuals (>85 years; n=6; age range, 86–91 years; 4 with known cerebrovascular disease). Sections were stained by Movat pentachrome method and chromogenic immunohistochemistry. All arteries between 20 and 1000 μm from each section were analyzed for vessel layer thickness (see online-only Data Supplement). We found no differences in layer thickness, inner diameter, or sclerotic index between frontal and temporal lobe vessels. Mouse monoclonal antibodies used included S100A4 (CPTC-S100A4-3; DSHB), smooth muscle actin (1A4; Dako), desmin (Dako), vimentin (Dako), anti-H (sc-59467; Santa Cruz Biotechnology), and LAMA2 (2D4; Abnova).

**Results**

Medial thickness was lower in CADASIL compared with controls and the very old (P<0.0001). The adventitia was not significantly increased in CADASIL compared with age-matched controls. Arterial diameters were not smaller in CADASIL compared with controls. Sclerotic index was significantly increased in CADASIL compared with other groups (P<0.00001). Intimal cells in CADASIL expressed smooth muscle actin, S100A4, and vimentin but not desmin.

**Conclusions**

—Principle changes of leptomeningeal arteries in CADASIL include intimal thickening and medial thinning, but not luminal narrowing. Smooth muscle–like cells participate in neointimal thickening of CADASIL arteries.

(Stroke. 2013;44:1456-1458.)

**Key Words:** CADASIL ■ cortex ■ leptomeningeal ■ neointimal hyperplasia ■ S100A4 ■ smooth muscle actin ■ vimentin
Table 1. Thickness of Arterial Layers in CADASIL, Control, and Aged Patients

<table>
<thead>
<tr>
<th></th>
<th>Intima</th>
<th>Media</th>
<th>Adventitia</th>
</tr>
</thead>
<tbody>
<tr>
<td>CADASIL</td>
<td>10.0±7.2*</td>
<td>5.2±6.2*</td>
<td>5.5±6.7</td>
</tr>
<tr>
<td>Control</td>
<td>1.9±1.7</td>
<td>6.9±5.0</td>
<td>4.9±7.1</td>
</tr>
<tr>
<td>Aged</td>
<td>2.1±1.5</td>
<td>6.3±4.9</td>
<td>4.1±5.7</td>
</tr>
</tbody>
</table>

Mean thickness (in micrometer) is given with SD. Number of arteries analyzed were 429 (CADASIL), 214 (control), and 313 (aged). CADASIL intima and media are significantly different from both controls and aged (*P<0.0001 for intima and P<0.01 for media). CADASIL indicates cerebral autosomal–dominant arteriopathy with subcortical infarcts and leukoencephalopathy.

Results

CADASIL arteries demonstrated notable intimal thickening and destruction of the vascular media. Of tunica thickness parameters (Table 1), intimal thickness of CADASIL vessels was the most dramatically distinguished from controls groups, with the average intima >5-fold thicker in CADASIL than the other groups. There was also a significant 22% to 35% attenuation of medial thickness in CADASIL compared with control groups. Adventitial thickness was not different in CADASIL compared with control groups. Total wall thickness was increased by >50% in CADASIL compared with controls and the aged (20.7 vs 13.8 and 12.5 μm).

CADASIL leptomeningeal luminal diameter was not different from that of controls (Table 2). The inner diameter: intima ratio was markedly decreased in CADASIL compared with other groups. The sclerotic index was increased in CADASIL vessels compared with both controls and the aged. Increases in intimal thickness and sclerotic index were also seen in perforating arteries of the CADASIL group (see online-only Data Supplement).

All CADASIL brains contained intimal cells with strong reactivity to smooth muscle actin and S100A4 and variable levels of vimentin (Figure). We did not observe desmin staining in intimal cells of any CADASIL samples. In many vessels, a fraction of cells expressed these markers, suggesting heterogeneity of cell phenotypes within the intima.

Discussion

We found that leptomeningeal arteries of CADASIL are substantially thickened, and thickening is predominantly a result of marked intimal hyperplasia that does not affect lumen diameter. Thickening of arteries is also seen in amyloid angiopathy, which, like CADASIL, is associated with a buildup of proteinaceous materials in small vessels. Intimal hyperplasia is a core vascular response found in diverse pathologies, including atherosclerosis, restenosis after angioplasty and stenting, and lacunar stroke. Genes responsible for each of these causes of intimal hyperplasia remain unknown. Thus, our studies demonstrate a novel, clear-cut monogenic cause (NOTCH3) of cerebral intimal hyperplasia.

CADASIL pathology includes cortical neuronal cell death, and patients experience symptoms, such as reversible encephalopathy, neuropsychiatric dysfunction, and epilepsy, which are usually attributed to cortical disease. In theory, leptomeningeal vascular changes could result in dysfunction of grey matter because this region of the brain is immediately downstream of large cortical arteries. However, lack of leptomeningeal luminal diameter changes in CADASIL suggests that vascular flow insufficiency may not explain cortical pathology. Our findings are congruent with studies that used high-field MRI to show that perforating arterial diameter was not different in CADASIL. Recent work has suggested that cortical damage results from injury to subcortical axons connected to neurons of the cortex.

We also demonstrate that cerebral intimal cells in CADASIL express a set of smooth muscle proteins (S100A4, smooth muscle actin, and vimentin) that are also frequently upregulated in fibroblasts, implicating the presence of dedifferentiated smooth muscle cells in brain vasculature. Proliferative intimal cells may be a common component of brain arterial disorders because cells with features similar to those described here have been found in a diverse range of diseases, including atherosclerosis, radiation necrosis, and moyamoya.

Thus, study of these cerebral intimal smooth muscle-like cells derived from CADASIL tissues may broadly impact other domains of cerebrovascular research.

Table 2. Lumen Diameter and SI in CADASIL, Control, and Aged Patients

<table>
<thead>
<tr>
<th></th>
<th>D-inner</th>
<th>SI</th>
<th>D-inner/Intima</th>
</tr>
</thead>
<tbody>
<tr>
<td>CADASIL</td>
<td>71.5±77.8</td>
<td>0.46±0.11</td>
<td>7.0±4.6*</td>
</tr>
<tr>
<td>Control</td>
<td>68.0±52.4</td>
<td>0.35±0.12</td>
<td>70.0±53.0</td>
</tr>
<tr>
<td>Aged</td>
<td>49.3±43.1</td>
<td>0.42±0.11</td>
<td>27.0±19.5</td>
</tr>
</tbody>
</table>

D-inner is presented in micrometers. SI was calculated as 1−[D-inner/D-outter] (mean±SD). CADASIL SI and D-inner/Intima are significantly different from controls and aged vessels (*P<0.0001). CADASIL indicates cerebral autosomal–dominant arteriopathy with subcortical infarcts and leukoencephalopathy; D-inner, inner diameter; and SI, sclerotic index.

Acknowledgments

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Disclosures

None.
References


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Supplemental Material

Advanced intimal hyperplasia without luminal narrowing
of leptomeningeal arteries in CADASIL

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SUPPLEMENTAL TABLE 1. Intimal thickness and sclerotic indices of perforating arteries in CADASIL, control, and aged patients.

<table>
<thead>
<tr>
<th></th>
<th>Intima</th>
<th>SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CADASIL</td>
<td>7.1+/4.7</td>
<td>0.45+/0.13</td>
</tr>
<tr>
<td>CONTROL</td>
<td>1.1+/0.7</td>
<td>0.34+/0.05</td>
</tr>
<tr>
<td>AGED</td>
<td>1.6+/1.7</td>
<td>0.38+/0.08</td>
</tr>
</tbody>
</table>

Mean thickness of the intima (in microns) is given by group. SI (sclerotic index) was calculated as 1-[D-inner/D-outer]. Means are given with standard deviations. All arteries of the white matter were analyzed in Movat pentachrome-stained sections. Unlike in leptomeningeal arteries, only the intimal layer of penetrating arteries could be definitively quantified. Number of arteries analyzed were 127 [CADASIL], 115 [control], and 104 [aged]. Emboldened CADASIL values are significantly different from both controls and aged vessels for both intimal thickness and SI (p<0.00001).
SUPPLEMENTAL METHODS.

Medial frontal lobe and anterior temporal lobe sections were chosen for examination because of predilection for the subcortical white matter of these areas to be affected in CADASIL. The cohort of CADASIL brains has been described before\(^1\). Control and aged samples were obtained from the Michigan Alzheimers Disease Center and the Maryland Brain Bank. All brain blocks were embedded in paraffin and sliced into 5 um sections. Sections were stained by Movat's pentachrome method. Briefly, this staining procedure was performed by sequential treatment with 1% Alcian blue, Verheoff's hematoxylin, 5% sodium thiosulfate, 0.08%/0.02% crocein scarlet/acid fuchsin, 5% phosphotungstic acid, and 6% safran. This method allows clear definition of the internal elastic lamina that forms the medial border of the intimal layer of arteries. Using this stain, the media and adventitia were distinguishable by a sharp color difference. All arteries between 20-1000 um from one frontal and one temporal lobe section were analyzed for each patient; arteries where the three layers were not clearly distinguishable or which were not complete or irregularly shaped were excluded from the study. Measurements were made on digitally captured images of arteries using Motic Images Plus 2.0. Two measurements for each layer were made at the narrowest point of each cross-sectioned vessel and averaged. All leptomeningeal arteries were combined from each patient group to determine the arterial parameters that included: intimal, medial, and adventitial thickness, inner diameter, and sclerotic index (SI). Parameters from all arteries from each group were compared by ANOVA, and significant differences were considered for \(p<0.01\).

Immunohistochemistry was performed by standard chromogenic methods on slides treated in boiling citrate for antigen retrieval\(^1\). Sections from each block were stained with mouse antibodies against H blood antigen (sc-59467; Santa Cruz Biotechnologies) and against LAMA2 (2D4; Abnova) which highlighted endothelial cells to confirm integrity of vascular tissue antigens in all sections from all patient groups.

Reference