Microvascular Structure and Network in the Retina of Patients With Ischemic Stroke

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Background and Purpose—Microvascular disease has been implicated in the pathogenesis of stroke. The retina provides a window to assess microcirculation noninvasively. We studied the association between quantitatively measured retinal microvascular characteristics and acute ischemic stroke.

Methods—We conducted a case-control study with acute ischemic stroke patients recruited from a tertiary hospital in Singapore and controls from the Singapore Epidemiology of Eye Disease program matched by 10-year age strata, sex, and race. Strokes were classified using modified Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria. Retinal vascular parameters were measured from retinal fundus photographs using a computer program. Logistic regression models for stroke were constructed adjusting for age, sex, race, and additionally for smoking, hypertension, diabetes mellitus, and hypercholesterolemia.

Results—We included 557 ischemic stroke cases (261 lacunar, 185 large artery, and 54 cardioembolic stroke) and 557 controls. After adjusting for vascular risk factors, decreased arteriolar fractal dimension (odds ratio [OR] per standard deviation [SD] decrease, 2.28; 95% confidence interval [CI], 1.80–2.87) and venular fractal dimension (OR per SD decrease, 1.80; 95% CI, 1.46–2.23), increased arteriolar tortuosity (OR per SD increase, 1.56; 95% CI, 1.25–1.95), and venular tortuosity (OR per SD increase, 1.49; 95% CI, 1.27–1.76), narrower arteriolar caliber (OR per SD decrease, 2.79; 95% CI, 2.21–3.53), and wider venular caliber (OR per SD increase, 1.57; 95% CI, 1.27–1.95) were associated with stroke. Stratification by stroke subtypes and further adjustment for retinopathy signs revealed similar results.

Conclusions—Patients with ischemic stroke have a sparser and more tortuous microvascular network in the retina. These findings provide insight into the structure and pattern of microcirculation changes in stroke.

Key Words: imaging ■ ischemic microvascular dysfunction ■ stroke
reflect advanced stages of structural microvascular damage, including breakdown of the blood–retina barrier.12,13 Advances in digital retinal photography and computer image analysis have now enabled more objective quantitative assessment of retinal microvascular structure and pattern, which may reflect earlier and subtler changes in the retinal microcirculation, even before retinopathy signs appear. Additionally, such assessments can be performed objectively even in the presence of retinopathy lesions. A range of novel retinal microvascular parameters have been proposed such as fractal dimension and tortuosity, which are global reflections of how optimal the retinal microcirculation is. To further test the hypothesis that microvascular changes may contribute to the pathogenesis of ischemic stroke, we focused our study on these novel retinal parameters.

In the present study, we applied this novel and advanced image analysis technology to measure and summarize the pattern and geometry of the retinal vasculature and examined their associations with ischemic stroke and its major subtypes.

Materials and Methods

Study Population

The Multi-Centre Retinal Stroke (MCRS) study is a cross-sectional observational study of patients with acute stroke, spanning 3 centers in Sydney, Melbourne, and Singapore.14 In the present study, only patients from Singapore were included. Patients presenting to the Singapore General Hospital with first-ever or recurrent stroke within 7 days of onset were recruited from 2005 to 2007. Patients were included if they were 40 to 80 years of age, of Chinese, Malay, or Indian race, had ischemic stroke pathogenesis, had adequate sitting ability to tolerate retinal photography, and had retinal photographs of gradable quality. Eligible stroke cases were matched to controls of the same 10-year age group, sex, and race, with no self-reported history of stroke selected from participants of the Singapore Epidemiology of Eye Diseases (SEED) study.15,16 This study is a population-based study of eye disease in an age-stratified random sample of Chinese, Malay, and Indian residents aged 40 to 80 years living in south western Singapore in the region where Singapore General Hospital provides stroke services. Written informed consent was obtained from each participant or next-of-kin, and previous approval for the project was obtained from the Singapore General Hospital’s Institutional Review Board.

Assessment of Retinal Vascular Parameters and Retinopathy

Retinal fundus photographs were taken of each eye with a nonmydriatic digital camera after dilation of pupils with 1% tropicamide eye drops according to a standardized protocol for participants of the MCRS study14 and the SEED studies.15,16 Optic disc–centered images of a randomly selected eye from each participant were masked and collated for centralized grading at the Singapore Eye Research Institute. Trained graders fed the images through a semiautomated computer-assisted program, Singapore I Vessel Assessment (version 3.0.0.0), grading to a standardized protocol, with measured area defined as 0.5 to 2.0 disc diameters away from the disc margin. Measurement of quantitative retinal parameters, which capture the overall structure of the retinal microvessels, was not influenced by the presence of localized retinopathy signs. Quantitative measures of the following retinal vascular parameters were extracted and used for analysis: retinal vascular caliber, fractal dimension, tortuosity, and branching angle.

Retinal Vascular Caliber

Retinal vascular caliber measurements were based on the revised Knudson-Parr-Hubbard formula, as described elsewhere,17 with arteriolar caliber summarized as central retinal arteriolar equivalent and venular caliber summarized as central retinal venular equivalent.

Retinal Vascular Fractal Dimension

Retinal vascular fractal dimension was calculated from skeletonized line tracing using the box counting method, a global measure summarizing the entire branching pattern of the retinal vascular tree.18 Larger values indicate a more complex branching pattern.

Retinal Vascular Tortuosity

Retinal vascular tortuosity was defined as the integral of the curvature square along the vessel path, normalized by the total path length.19 Estimates were summarized as retinal arteriolar tortuosity and retinal venular tortuosity. Smaller values indicate straighter vessels.

Retinal Vascular Branching Angle

Branching angle is defined as the first angle subtended between 2 daughter vessels at each vascular bifurcation.20 The average branching angle of the arterioles and venules of the eye were calculated.

Assessment of Stroke and Stroke Subtypes

All stroke patients were assessed by a standardized questionnaire with an interview conducted by trained medical staff, a neurological examination, and brain imaging (computed tomography or MRI). Stroke was classified at clinical consensus meetings with research staff and clinicians using a modified version of the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification.21 Stroke subtypes were defined as large-vessel atherosclerotic stroke, small-vessel lacunar stroke, cardioembolic stroke, stroke of other pathogenesis, and stroke of undetermined pathogenesis. Because the original TOAST classification would lead to a large proportion of strokes with uncertain classification, a pragmatic modification was adopted from the Greater Metropolitan Clinical Task Force for Stroke in New South Wales. Additionally, references to hypertension and diabetes mellitus as risk factors for lacunar stroke also were removed to avoid risk factor bias in classification, resulting in a clinico-neuroanatomical definition. Further details of classification have been published elsewhere.10,14

Assessment of Cardiovascular Risk Factors

Both patients with ischemic stroke and control participants from the SEED study (and their caregivers) were administered detailed questionnaires for information on smoking and history of physician-diagnosed or medication use for hypertension, diabetes mellitus, and hypercholesterolemia. Additionally, as part of clinical care for stroke, patients with ischemic stroke underwent standard clinical assessment after the acute phase for stroke to determine cardiovascular risk factors; this included multiple blood pressure measurements and fasting blood samples for glucose, hemoglobin A1C, and lipids. Controls, as part of the SEED study, underwent extensive examination on the day of retinal photography, which included ≥2 blood pressure measurements at the same sitting and random blood samples for glucose, hemoglobin A1C, and cholesterol. Hypertension was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg at examination, a reported history of physician-diagnosed
hypertension or a self-reported history of antihypertensive medication use, or both. Diabetes mellitus was defined as random blood glucose ≥200 mg/dL (11.1 mmol/L), fasting blood glucose ≥126 mg/dL (7.0 mmol/L), hemoglobin A1C ≥7%, self-reported history of physician-diagnosed diabetes mellitus, or self-reported history of antihyperglycemic medication use. Hypercholesterolemia was defined as total cholesterol ≥6.2 mmol/L, or a self-reported history of physician-diagnosed hypercholesterolemia, or self-reported use of lipid-lowering medication use.

**Statistical Analysis**
Baseline characteristics, prevalence of cardiovascular risk factors, and retinal geometric parameters were compared between cases and controls using Student t test for continuous variables and Pearson χ² test for categorical variables. Odds ratios and their 95% confidence intervals for stroke and its subtypes per SD increase or decrease in retinal vascular parameters were calculated. We initially adjusted for age, sex, and race, and additionally for hypertension, diabetes mellitus, hypercholesterolemia, and smoking history. As proposed previously, because of the high correlation between arteriolar and venular caliber values (Pearson correlation coefficient, 0.691; P<0.001), all models testing vessel caliber were additionally adjusted for the companion vessel. Finally, all fully adjusted models were additionally adjusted for the presence of retinopathy signs to examine whether the associations between novel retinal parameters and stroke were independent of retinopathy signs. All analyses were performed using SPSS version 17.0.

**Results**
Among the patients recruited into the Singapore component of the MCRS study, a total of 557 patients were eligible for inclusion into this study and 557 controls were subsequently selected from the SEED study cohorts by matching for sex, 10-year age group, and race. Comparisons of baseline characteristics between ischemic stroke cases and controls are presented in Table 1. In general, patients with ischemic stroke were more likely to be current smokers and to have hypertension, diabetes mellitus, and hypercholesterolemia. The most common stroke subtype was lacunar infarction (n=261; 47.0%), followed by large-artery stroke (n=185; 33.3%) and cardioembolic stroke (n=54; 9.7%), whereas the remaining 56 (10.1%) strokes were of other or undetermined causes.

As shown in Table 1 and in Figures 1 and 2, patients with ischemic stroke had relatively smaller fractal dimensions (total, arteriolar, and venular), higher arteriolar and venular tortuosity, and narrower arteriolar and venular calibers. After adjustment for age, sex, race, and for the cardiovascular risk factors of hypertension, diabetes mellitus, hypercholesterolemia, and smoking history (Table 2), decreasing arteriolar and venular fractal dimension, increasing arteriolar and venular tortuosity, narrower arteriolar caliber, and wider venular caliber were associated with ischemic stroke, whereas associations with other branching parameters such as branching angle were not significant.

Associations of these parameters were similar with stroke subtypes of lacunar, large artery, and cardioembolic stroke, with no observable differences in effect sizes between different stroke pathogeneses (Table 2). Furthermore, when fully adjusted models were additionally adjusted for presence of clinically visible retinopathy signs, decreasing arteriolar and venular fractal dimension, increasing arteriolar and venular tortuosity, narrower arteriolar caliber, and wider venular caliber were associated with ischemic stroke, whereas associations with other branching parameters such as branching angle were not significant.

### Table 1. Comparison of Baseline Characteristics and Retinal Geometric Parameters in Stroke Cases and Controls

<table>
<thead>
<tr>
<th>Demographics and cardiovascular risk factors</th>
<th>Cases (n=557)</th>
<th>Controls (n=557)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td>356 (63.9)</td>
<td>356 (63.9)</td>
<td>1.000</td>
</tr>
<tr>
<td>Age (years), mean (SD)</td>
<td>61.9 (9.4)</td>
<td>61.9 (9.1)</td>
<td>0.988</td>
</tr>
<tr>
<td>Chinese race, n (%)</td>
<td>451 (81.0)</td>
<td>451 (81.0)</td>
<td>1.000</td>
</tr>
<tr>
<td>Malay race, n (%)</td>
<td>63 (11.3)</td>
<td>63 (11.3)</td>
<td></td>
</tr>
<tr>
<td>Indian race, n (%)</td>
<td>43 (7.7)</td>
<td>43 (7.7)</td>
<td></td>
</tr>
<tr>
<td>Never smoker, n (%)</td>
<td>274 (50.9)</td>
<td>396 (71.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ex-smoker, n (%)</td>
<td>92 (17.1)</td>
<td>112 (20.1)</td>
<td></td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>163 (30.3)</td>
<td>49 (8.8)</td>
<td></td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>377 (67.8)</td>
<td>235 (42.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>226 (40.6)</td>
<td>60 (10.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypercholesterolemia, n (%)</td>
<td>259 (46.5)</td>
<td>219 (39.7)</td>
<td>0.025</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Retinal vessel parameters, mean (95% confidence interval)</th>
<th>Cases (n=557)</th>
<th>Controls (n=557)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total fractal dimension</td>
<td>1.379 (1.373–1.385)</td>
<td>1.421 (1.416–1.425)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Arteriolar fractal dimension</td>
<td>1.153 (1.147–1.159)</td>
<td>1.198 (1.193–1.203)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Venular fractal dimension</td>
<td>1.185 (1.179–1.190)</td>
<td>1.208 (1.204–1.213)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Arteriolar curvature tortuosity (×10⁴)</td>
<td>0.646 (0.622–0.670)</td>
<td>0.605 (0.592–0.618)</td>
<td>0.003</td>
</tr>
<tr>
<td>Venular curvature tortuosity (×10⁴)</td>
<td>0.852 (0.835–0.869)</td>
<td>0.785 (0.769–0.802)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Arteriolar caliber</td>
<td>127.8 (126.7–128.8)</td>
<td>134.5 (133.1–135.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Venular caliber</td>
<td>198.6 (197.0–200.2)</td>
<td>199.4 (197.4–201.4)</td>
<td>0.564</td>
</tr>
<tr>
<td>Arteriolar branching angle (°)</td>
<td>75.2 (74.1–76.3)</td>
<td>75.7 (74.8–76.6)</td>
<td>0.511</td>
</tr>
<tr>
<td>Venular branching angle (°)</td>
<td>77.5 (76.4–78.6)</td>
<td>77.8 (76.8–78.7)</td>
<td>0.720</td>
</tr>
<tr>
<td>Presence of retinopathy signs, n (%)</td>
<td>416 (74.7)</td>
<td>56 (10.1)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Discussion

In this study, we demonstrate for the first time to our knowledge that the structure and pattern of quantitatively measured microvasculature in the retina are different in patients with ischemic stroke compared with healthy control subjects. Novel retinal measures indicative of a sparser and more tortuous vascular network, in addition to narrower arteriolar caliber and wider venular caliber, were associated with ischemic stroke and with its major etiologic subtypes, independent of traditional cardiovascular risk factors and clinically visible retinopathy signs.

Several studies previously have suggested that cerebral small-vessel disease is implicated in ischemic stroke and is considered to be highly related to, but not restricted to, lacunar infarction. Pathological processes in the brain such as atherosclerosis, lipohyalinosis, and arteriosclerosis lead to the destruction and occlusion of small perforating vessels and cause vessel wall remodeling, resulting in elongated and tortuous vessels associated with increased vascular leakage. These changes affect the ability of cerebral arterioles to maintain control of local blood flow, predisposing areas served by these dysfunctional vessels to ischemic damage. In the retina, vessel rarefaction and collapse, leading to reduction in vascular fractal dimension, is associated with hypoxia; whereas increased vessel tortuosity is indicative of vessel wall dysfunction and blood–retina barrier damage. Our findings suggest that similar pathological changes in the small vessels of the brain and retina may adversely affect perfusion and vessel wall function. Our study provides direct in vivo data that show a difference in the pattern and structure of the microvascular network between patients with stroke compared with healthy controls.

To our knowledge, this is the first study of a comprehensive assessment of quantitatively measured retinal microvascular parameters in stroke cases. Previous studies have identified individual associations with specific parameters. For example, the link between retinal fractal dimension and stroke has been reported. Our study shows that reduced retinal fractal dimension and increased retinal vessel tortuosity is associated with ischemic stroke, suggesting that the retinal vascular network can reflect corresponding microvascular abnormalities indicative of reduced vascular perfusion and barrier damage leading to ischemic damage in the cerebral vasculature. Furthermore, because these morphological changes in the retinal microvasculature are similarly associated with both large-artery stroke and lacunar infarcts in our study, it is suggested that structural changes to retinal microvessels not only reflect small-vessel pathology but also may result from downstream effects of large-artery pathology in the retinal and cerebral circulations.

Importantly, it has been demonstrated that similar changes in retinal pattern and geometry are also implicated in cognitive dysfunction and dementia (Alzheimer disease; Carol Yim Cheung, PhD, unpublished data, 2013), suggesting that similar cerebral microvascular pathologies—as reflected by alterations in the retinal microvasculature—may underlie both stroke and dementia.

The most studied parameter is retinal vascular caliber, and our study is consistent with previous population-based studies that show narrower arteriolar caliber and wider venular caliber are associated with ischemic stroke. However, because of pulse period variation in vessel calibers, measurements from a single time point may not be as useful in stroke risk
stratification, and less time-variable novel structural retinal parameters may be more informative in evaluating cerebral microvascular damage. Fractals and tortuosity measures are attractive because they are relatively static and reflect blood distribution optimality and efficiency; in our study, we found that subtle changes measured by these parameters remain associated with stroke even after adjustment for visible retinopathy signs. Hence, these novel structural parameters have potential advantages beyond that of qualitative signs. These definitely should be further validated and studied in prospective studies.

Several limitations need to be discussed. First, because we had no data on hemorrhagic strokes, we could only focus on ischemic stroke. Second, because patients were required to sit upright for retinal photography, we were limited to patients with relatively mild strokes. Third, the definition of

Table 2. Age, Sex, Race, and Multivariable-Adjusted ORs of Retinal Vascular Parameters for Stroke and its Subtypes

<table>
<thead>
<tr>
<th>Retinal Vessel Parameters</th>
<th>Stroke* (n=557)</th>
<th>Stroke† (n=557)</th>
<th>Stroke‡ (n=557)</th>
<th>Subtypes by TOAST Classification†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fractal dimension</td>
<td></td>
<td></td>
<td></td>
<td>Lacunar (n=261)</td>
</tr>
<tr>
<td>Arteriolar per SD decrease</td>
<td>2.44 (2.09–2.87)</td>
<td>2.28 (1.80–2.87)</td>
<td>1.92 (1.47–2.51)</td>
<td>2.28 (1.80–2.87)</td>
</tr>
<tr>
<td>Venular per SD decrease</td>
<td>1.63 (1.42–1.87)</td>
<td>1.80 (1.46–2.23)</td>
<td>1.79 (1.39–2.31)</td>
<td>1.80 (1.46–2.23)</td>
</tr>
<tr>
<td>Tortuosity</td>
<td></td>
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<td></td>
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<tr>
<td>Arteriolar per SD increase</td>
<td>1.29 (1.09–1.53)</td>
<td>1.56 (1.25–1.95)</td>
<td>1.91 (1.32–2.76)</td>
<td>1.45 (1.13–1.86)</td>
</tr>
<tr>
<td>Venular per SD increase</td>
<td>1.50 (1.31–1.73)</td>
<td>1.49 (1.27–1.76)</td>
<td>1.32 (1.02–1.72)</td>
<td>1.45 (1.21–1.75)</td>
</tr>
<tr>
<td>Caliber</td>
<td></td>
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<tr>
<td>Arteriolar§ per SD decrease</td>
<td>2.61 (2.14–3.17)</td>
<td>2.79 (2.21–3.53)</td>
<td>2.35 (1.64–3.35)</td>
<td>2.87 (2.19–3.76)</td>
</tr>
<tr>
<td>Venular§ per SD increase</td>
<td>1.83 (1.53–2.20)</td>
<td>1.57 (1.27–1.95)</td>
<td>1.34 (0.96–1.88)</td>
<td>1.60 (1.25–2.06)</td>
</tr>
<tr>
<td>Branching angle</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Arteriolar, per SD increase</td>
<td>0.96 (0.95–1.08)</td>
<td>1.08 (0.94–1.24)</td>
<td>1.24 (0.98–1.58)</td>
<td>1.07 (0.90–1.27)</td>
</tr>
<tr>
<td>Venular, per SD increase</td>
<td>0.98 (0.87–1.10)</td>
<td>1.00 (0.87–1.15)</td>
<td>1.12 (0.91–1.39)</td>
<td>0.98 (0.83–1.15)</td>
</tr>
</tbody>
</table>

CI indicates confidence interval; OR, odds ratio; SD, standard deviation; and TOAST, Trial of Org 10172 in Acute Stroke Treatment.

*Adjusted for age, sex, race, and arteriolar and venular caliber.
†Adjusted for age, sex, race, smoking status, hypertension status, diabetes mellitus status, and hypercholesterolemia status.
‡Adjusted for age, sex, race, smoking status, hypertension status, diabetes mellitus status, hypercholesterolemia status, and retinopathy signs.
§Additionally adjusted for other vessel caliber.
cardiovascular risk factors was not exactly similar in cases (fasting blood samples) and controls (random blood samples), and confounding may not have been fully accounted for. Fourth, although Singapore General Hospital provides stroke services to a region overlapping the SEED catchment area, some stroke cases may be from outside this catchment area. However, because Singapore is a small city state with a homogeneously urban environment, the SEED region is not expected to be significantly different from that of the rest of Singapore. Finally, the case-control design of our current study did not allow us to determine the temporal sequence of these associations. Further longitudinal studies are required to establish whether changes in retinal vascular geometry are related to the risk of incident stroke and whether they are indicative of cerebrovascular risk beyond conventional risk indicators. Strengths of our study include the large sample size, population-matched controls, and objective measurement of quantitative retinal vascular parameters.

Conclusions

Retinal vascular changes reflective of a sparser and more tortuous network in the microcirculation are seen in patients with ischemic stroke, beyond the effects of age, vascular risk factors, and clinically visible retinopathy signs. These data provide convincing evidence of global alterations in the retinal microvasculature in stroke. More prospective studies are required to confirm these associations before they can be implemented as a screening tool.

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Disclosures

None.

References

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