Changes in the retinal vasculature are established biomarkers of diabetic retinopathy (DR). In particular, the associations with measurements of retinal vessel caliber have been extensively studied in several large epidemiologic studies. Daxer described the analysis of retinal vascular fractal dimension in eyes with DR. Since then, computer-based programs have been developed to perform quantitative assessment of not only the fractal dimensions, but also a whole new class of geometric branching network parameters of the retinal vasculature. The vascular network is believed to be organized to minimize shear stresses and work across the system, and these parameters may provide an indication of how closely a given network conforms to the geometric ideal. In contrast to retinal vessel caliber measurements, which are generally performed at a fixed location relative to the optic disc and which measure only a single property of the retinal vessels, geometric network measures may provide a more global indication of the overall health of the vascular system.

Higher retinal vascular fractal dimension, indicating a more complex retinal vascular network, has been shown to be associated with diabetes. Data from cross-sectional as well as prospective studies on retinal vessel geometry and DR have yielded inconsistent results. Some studies have found no associations with these measurements, while others have found associations between DR and lower fractal dimensions. A study by Talu found that the fractal dimensions were higher in eyes with mild DR, but lower in eyes with moderate or severe DR. These inconsistent findings have been attributed to differences in study design, population ethnicity, as well as possibly the type of diabetes.

The aim of this study was to analyze the associations between retinal vessel geometry measures and the 1-year incidence and progression of DR in a Chinese population. We hypothesized that changes in the retinal vessel geometry may predict DR incidence or progression.

**METHODS.** This was a prospectively designed cohort study of adult subjects with diabetes mellitus. Retinal vascular geometry was quantified from fundus photographs using a semiautomated computer-assisted program. Diabetes retinopathy was graded from retinal photographs at baseline and 1 year. Incident DR and 2-step change in DR were analyzed.

**RESULTS.** In total, 249 subjects were included. Their mean age was 59.9 ± 8.9 years, 74% were male, and the mean glycated hemoglobin A1c (HbA1C) and duration of diabetes were 7.7 ± 1.4% and 14.3 ± 10.6 years, respectively. The distribution of DR severity at baseline was no DR in 35.7%, minimal nonproliferative diabetic retinopathy (NPDR) in 15.3%, mild NPDR in 14.6%, moderate NPDR in 23.1%, severe NPDR in 5.1%, and proliferative DR in 6.1% of eyes. In multivariate analyses adjusting for age, duration of diabetes, sex, smoking status, HbA1C, hypertension, and hyperlipidemia, subjects with higher venular fractal dimensions were more likely to have incident DR (odds ratio [OR] 0.38, [95% confidence interval (CI), 0.15–0.96], \( P = 0.032 \), per SD decrease). Lower venular tortuosity was associated with a lower likelihood of DR progression (OR 0.76, [95% CI, 0.59–0.97], \( P = 0.005 \), per SD decrease). Lower arteriolar tortuosity was associated with a greater likelihood of DR regression (OR 1.95, [95% CI 1.07–3.56], \( P = 0.037 \), per SD decrease).

**CONCLUSIONS.** Novel measures of retinal vascular geometry are associated with the incidence and progression of DR at 1 year. These geometric measures are likely to represent early dysfunction in the retinal microvasculature.

**Keywords:** diabetic retinopathy, retinal vasculature, epidemiology
relevant sociodemographic data and medical history at the baseline visit. Data collected included country and state of birth, marital status, education, occupation and current housing status, participants’ lifestyle factors, history of smoking, eye symptoms, use of spectacles, falls and fractures, current medications, systemic medical and surgical history, and family history of eye diseases.

Fasting venous blood sample were collected for biochemistry tests including serum lipids (total cholesterol, high-density lipid [HDL], low-density lipid [LDL] cholesterol), glycated hemoglobin A1c (HbA1C), creatinine, and glucose.

All study procedures were performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from the subjects, and the institutional review board of the Singapore Eye Research Institute approved the study.

Assessment of Diabetic Retinopathy
Retinal photography was performed following a standardized protocol as described in other publications from our center. After pupil dilation using tropicamide 1% and phenylephrine hydrochloride 2.5%, two retinal photographs, centered at the optic disc and macula, were obtained from each eye of the participants using a digital retinal camera (Canon CR-DGi with a 10-D SLR back; Canon, Tokyo, Japan). Trained, masked graders at the Singapore Eye Research Institute then graded photographs.

Diabetic retinopathy was considered present if any characteristic lesion as defined by the Early Treatment Diabetic Retinopathy Study (ETDRS) severity scale was present: microaneurysms, hemorrhages, cotton wool spots, intraretinal microvascular abnormalities, hard exudates, venous beading, and new vessels. For each eye, a retinopathy severity score was assigned according to a scale modified from the Airlie House classification system. The DR level for each participant was then derived by concatenating the levels for the two eyes, giving greater weightage to the eye with the more severe grade. This grading scheme has 15 levels of severity. If an eye was ungradable, it was presumed to have an equivalent grade to the fellow eye.

The main outcomes were defined as follows. Incident DR was defined as the presence of any DR (level 21/21 or worse) at 1 year in subjects with no DR (level 10/10) at baseline. Diabetic retinopathy progression was defined as any two level or greater worsening of DR at 1 year, while DR regression was any two level or greater improvement in DR at 1 year.

Measurement of Retinal Vascular Network Geometry
Retinal vessel network geometry was measured from digital fundus photographs using methods described in our previous publications. Two retinal images were obtained from each eye using the same fundus camera for DR assessment, one centered on the optic disc and another centered on the fovea. We used an optic disc-centered photograph of the right eye of each participant; if the photograph of the right eye was ungradable, the measurement was performed on the left eye.

We used a locally developed, semiautomated computer-assisted program (Singapore I Vessel Assessment [SIVA], version 3.0; Singapore Eye Research, Singapore) to quantitatively measure a range of retinal vascular parameters, including retinal vascular caliber, retinal vascular tortuosity, and retinal branching measures, from the images. Trained and masked graders used the SIVA program to measure the retinal vasculature, according to a standardized protocol. Images of poor quality, including those due to media opacities (e.g., dense lens opacity), small pupils, or image defocus were excluded. There were two graders involved in this study. The interobserver reliability was 0.76 (95% confidence interval [CI] 0.65–0.87).

The SIVA program automatically identifies the optic disc, projects a grid referenced to the optic disc, identifies the vessel types (arterioles and venules), and performs the vessel measurements detailed below. The measured area of retinal vascular tortuosity and branching measures was standardized as the region from 0.5 to 2.0 disc diameters from the disc margin. Trained graders performed visual evaluations of the automated measurements with manual corrections if necessary. Retinal vascular tortuosity is defined as the integral of the curvature square along the path of the vessel, normalized by the total path length. All vessels coursing through the measured zone with a width larger than 40 μm were measured. These measures do not have units, and a smaller tortuosity value indicates a straighter vessel. The estimates were summarized as the average retinal arteriolar tortuosity and retinal venular tortuosity of the eye. Retinal vascular fractal dimension was calculated from the skeletonized line tracing using the box-counting method, a “global” measure summarizing the whole branching pattern of the retinal vascular tree. Retinal branching angle (BA) (ω0), which is the sum of ω1 + ω2, ω1 < ω2, which is defined as the angle subtended between two daughter vessels at each vascular bifurcation. The mean widths of the parent vessel (d0) and the two daughter branching vessels (d1 and d2, d1 < d2) were also measured. Retinal branching coefficient (BC) was defined as the sum of the square of the two branching vessel widths divided by the square of the parent vessel width (i.e., d12 + d22/d02). All vessels with the first bifurcation within the measured zone were measured.

Statistical Analyses
Descriptive data are presented as mean (SD) for continuous variables or number (percentages) of participants for categorical variables. Multivariable cox proportional models were constructed with the DR outcome as the dependent variables to examine odds ratios (ORs) with 95% CI between arteriolar and venular dilation. Initial adjustments were made for age and sex. Smoking, mean duration of diabetes, glycated hemoglobin, hypertension, and hyperlipidemia were added in a second multivariate model. Proportional hazard assumption was confirmed for all predictors with Schoenfeld’s residuals.

We regarded P values of less than 0.05 from two-sided tests as statistically significant. All statistical analyses were performed using STATA (Version 12; StataCorp, College Station, TX, USA).

RESULTS
In total, 249 subjects were included. The mean age of the subjects was 59.9 ± 8.9 years, the majority was male (74%) and the mean HbA1c level and mean duration of diabetes were 7.7 ± 1.4% and 14.3 ± 10.6 years, respectively. There were 15 (6%) subjects with type 1 diabetes. All of the type 1 diabetics were on insulin therapy, while 48 of the type 2 diabetics were receiving insulin (Table 1).

The distribution of DR severity at baseline was no DR in 35.7%, minimal nonproliferative diabetic retinopathy (NPDR) in 15.3%, mild NPDR in 14.6%, moderate NPDR in 23.1%, severe NPDR in 5.1%, and proliferative DR in 6.1% of eyes.

The incidence of DR at 1 year was 19 of 90 subjects (21.1%) and the incidence of PDR was 8 of 235 subjects (3.4%). The number of subjects with 2-step or greater progression of DR at 1 year was 58 of 235 (16.2%), and the number of subjects with...
2-step or greater regression of DR at 1 year was 51 of 143 subjects (35.7%; Table 1).

When stratified by quartiles, in univariate analyses there were no associations between incident DR and the venular fractal dimension. However, in multivariate analyses adjusting for age, sex, smoking status, HbA1c, hypertension, and hyperlipidemia, subjects with the highest venular fractal dimensions were more likely to have incident DR (OR 0.38, [95% CI, 0.15–0.96], \(P = 0.032\), per SD decrease). There were no significant

**Table 1.** Baseline Characteristics of Subjects by DR Outcomes at 1 Year

<table>
<thead>
<tr>
<th></th>
<th>Whole Cohort</th>
<th>Subjects With Incident DR, (n = 19/N = 90)</th>
<th>Subjects With Incident PDR, (n = 8/N = 235)</th>
<th>Subjects With DR Progression, (n = 38/N = 235)</th>
<th>Subjects With DR Regression, (n = 51/N = 143)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (y)</td>
<td>59.9 (8.9)</td>
<td>66.2 (5.4)</td>
<td>61.3 (9.3)</td>
<td>61.1 (8.8)</td>
<td>57.5 (8.7)</td>
</tr>
<tr>
<td>Mean duration of diabetes (y)</td>
<td>14.3 (10.6)</td>
<td>11.7 (10.4)</td>
<td>16.9 (13.1)</td>
<td>14.2 (10.8)</td>
<td>15.0 (10.0)</td>
</tr>
<tr>
<td>Mean HbA1c (%)</td>
<td>7.70 (1.40)</td>
<td>7.33 (0.96)</td>
<td>8.84 (1.36)</td>
<td>8.03 (1.37)</td>
<td>7.93 (1.48)</td>
</tr>
<tr>
<td>Sex, Female (%)</td>
<td>66 (26.5)</td>
<td>5 (26.3)</td>
<td>3 (37.5)</td>
<td>11 (29.0)</td>
<td>13 (25.5)</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>166 (66.7)</td>
<td>12 (63.2)</td>
<td>4 (50.0)</td>
<td>22 (57.9)</td>
<td>38 (74.5)</td>
</tr>
<tr>
<td>Current</td>
<td>30 (12.1)</td>
<td>2 (10.5)</td>
<td>2 (25.0)</td>
<td>5 (13.2)</td>
<td>2 (3.9)</td>
</tr>
<tr>
<td>Past</td>
<td>53 (21.3)</td>
<td>5 (26.3)</td>
<td>2 (25.0)</td>
<td>11 (29.0)</td>
<td>11 (21.6)</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>166 (66.7)</td>
<td>11 (57.9)</td>
<td>4 (50.0)</td>
<td>26 (68.4)</td>
<td>35 (68.6)</td>
</tr>
<tr>
<td>Hyperlipidemia (%)</td>
<td>187 (76.0)</td>
<td>16 (84.2)</td>
<td>6 (75.0)</td>
<td>31 (81.6)</td>
<td>37 (74.0)</td>
</tr>
</tbody>
</table>

**Table 2.** Associations Between Changes in Retinal Vessel Geometry and Diabetic Retinopathy Incidence and Progression

<table>
<thead>
<tr>
<th></th>
<th>Incident DR</th>
<th>DR Progression</th>
<th>DR Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Univariate, HR (95% CI)</td>
<td>Multivariate, * HR (95% CI)</td>
<td>Univariate, HR (95% CI)</td>
</tr>
<tr>
<td>Fractal dimension arteriole</td>
<td>0.67 (0.35, 1.31)</td>
<td>0.39 (0.14, 1.10)</td>
<td>0.99 (0.72, 1.34)</td>
</tr>
<tr>
<td>Fractal dimension arteriole per SD decrease</td>
<td>0.42</td>
<td>0.1</td>
<td>0.69</td>
</tr>
<tr>
<td>Fractal dimension venule</td>
<td>0.68 (0.38, 1.21)</td>
<td>0.38 (0.15, 0.96)</td>
<td>0.85 (0.59, 1.21)</td>
</tr>
<tr>
<td>Fractal dimension venule per SD decrease</td>
<td>0.21</td>
<td>0.032</td>
<td>0.20</td>
</tr>
<tr>
<td>Curvature turtosity arteriole</td>
<td>1.70 (0.87, 3.34)</td>
<td>1.52 (0.71, 3.27)</td>
<td>0.98 (0.79, 1.23)</td>
</tr>
<tr>
<td>Curvature turtosity arteriole per SD decrease</td>
<td>0.16</td>
<td>0.43</td>
<td>0.26</td>
</tr>
<tr>
<td>Curvature turtosity venule</td>
<td>1.26 (0.73, 2.19)</td>
<td>1.07 (0.60, 1.92)</td>
<td>0.96 (0.78, 1.17)</td>
</tr>
<tr>
<td>Curvature turtosity venule per SD decrease</td>
<td>0.51</td>
<td>0.59</td>
<td>0.026</td>
</tr>
<tr>
<td>Branching coefficient arteriole</td>
<td>1.42 (0.71, 2.83)</td>
<td>1.36 (0.65, 2.85)</td>
<td>1.04 (0.71, 1.53)</td>
</tr>
<tr>
<td>Branching coefficient arteriole per SD decrease</td>
<td>0.49</td>
<td>0.53</td>
<td>0.26</td>
</tr>
<tr>
<td>Branching coefficient venule</td>
<td>1.04 (0.64, 1.70)</td>
<td>0.95 (0.55, 1.65)</td>
<td>1.27 (0.68, 2.37)</td>
</tr>
<tr>
<td>Branching coefficient venule per SD decrease</td>
<td>0.56</td>
<td>0.97</td>
<td>0.038</td>
</tr>
<tr>
<td>Branching angle arteriole</td>
<td>1.08 (0.65, 1.77)</td>
<td>1.35 (0.67, 2.70)</td>
<td>1.36 (0.99, 1.89)</td>
</tr>
<tr>
<td>Branching angle arteriole per SD decrease</td>
<td>0.64</td>
<td>0.33</td>
<td>0.089</td>
</tr>
<tr>
<td>Branching angle venule</td>
<td>1.22 (0.75, 2.00)</td>
<td>1.78 (0.80, 3.97)</td>
<td>0.96 (0.73, 1.27)</td>
</tr>
<tr>
<td>Branching angle venule per SD decrease</td>
<td>0.80</td>
<td>0.22</td>
<td>0.46</td>
</tr>
</tbody>
</table>

* Adjusted for age, sex, smoking status, HbA1c, hypertension, and hyperlipidemia. HR, harzard ratio.
associations between incident DR and any of the other retinal vessel measures (Table 2).

For the outcome of 2-step DR progression, higher venular tortuosity was associated with a greater likelihood of DR progression in univariate analyses (P = 0.026). These associations persisted in multivariate analyses adjusting for age, sex, smoking status, Hba1c, hypertension, and hyperlipidemia (OR 0.76, [95% CI, 0.59–0.97], P = 0.03, per SD decrease). There were no significant associations between DR regression and any of the other retinal vessel measures (Tables 2, 3).

For the outcome of 2-step DR regression, lower arteriolar tortuosity was associated with a greater likelihood of DR regression in univariate analyses (P = 0.024). These associations persisted in multivariate analyses adjusting for age, sex, smoking status, Hba1c, hypertension, and hyperlipidemia (OR 1.95, [95% CI, 1.07–3.56], P = 0.037, per SD decrease). There were no significant associations between DR regression and any of the other retinal vessel measures (Tables 2, 3).

A representative case is shown in the Figure.

DISCUSSION

Our study presents novel prospective data on the associations between retinal vessel geometry and DR incidence and progression over 1 year. Larger retinal venular fractal dimensions are associated with increased likelihood of DR incidence. Higher venular tortuosity is associated with greater likelihood of DR progression, while lower arteriolar tortuosity is associated with greater likelihood of DR regression.

Data on the relationships between retinal vessel geometry and diabetes and DR have been inconsistent. In patients with diabetes but no DR, data from the Australian Diabetes, Obesity, and Lifestyle (AusDiab) Study as well as from India have shown that retinal vessel fractal dimensions are higher in patients with diabetes than in those without diabetes.

Data from studies on DR have come from both cross-sectional and prospective studies. Talu compared 24 healthy subjects with 148 subjects with various grades of DR. Healthy subjects had lower fractal dimensions than mild DR subjects, but higher fractal dimensions than subjects with moderate or severe DR. Cheung performed a cross-sectional study on a cohort of type 1 diabetic patients and reported higher fractal dimensions in eyes with early DR signs. However, in the Singapore Malay Eye Study (SIMES), diabetes was associated with less tortuous arterioles, but DR was not associated with retinal tortuosity. Likewise, Kunicki reported that fractal analyses were not sensitive enough to differentiate eyes with mild DR and no DR. Prospective data from a hospital based study of adolescents with type 1 diabetes found that the fractal dimensions did not predict the 3-year incidence of DR. The precise interpretation of fractal dimensions is currently unclear. It has been suggested that an ideal or optimal fractal dimension may not lie at either end of a linear spectrum. For example, a study on the association between the retinal vascular fractal dimension and chronic kidney disease found a U-shaped relationship, in which subjects in the highest and lowest quintiles of fractal dimensions had higher prevalence of kidney disease.

In our current study,
larger retinal venular but not arteriolar fractal dimensions were associated with higher DR incidence at 1 year. A larger fractal dimension is believed to represent greater overall complexity of the vascular tree, and the association we found may reflect greater arterio-venous shunting in cases that develop DR.

Data on retinal vascular tortuosity have been limited. In the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT), no association was found between tortuosity and DR. In a clinical case study of 224 patients with diabetes and 103 nondiabetic controls, arteriolar tortuosity was associated with mild and moderate nonproliferative DR but not more severe grades of DR. There were no associations with venular tortuosity. In our study, greater venular tortuosity was associated with DR progression, and, conversely, greater arteriolar tortuosity was associated with a lower likelihood of DR regression. Increased venular tortuosity is a well-established clinical sign in DR, and various mechanisms have been postulated to account for this including impaired autoregulation and hemodynamic shear stress, as well as VEGF-induced changes. That less tortuous arterioles were associated with a greater likelihood of DR regression suggests that lower arteriolar tortuosity may represent a healthier vascular tree. It has been shown that early diabetes is associated with retinal arteriolar vasoconstriction, which may in turn appear as less tortuous arterioles. Persistent exposure to hyperglycemia may lead to subsequent pericyte loss and permanent arteriolar dilation. As such, less tortuous arterioles may represent earlier and potentially reversible microvascular changes of DR.

The strengths of our study include a relatively large clinical cohort, and standardized assessments of DR severity from retinal photography as well as systemic covariates. Our study also provides novel prospective data with high compliance with follow-up. The SIVA software we used, while relatively new, has been extensively used in numerous publications, and all graders were masked to patient characteristics. General limitations of our study include the possibility of loss to follow-up bias, as well as selection bias and limited generalizability of our results as subjects were drawn from a hospital-based population. This may account for the relatively high 1-year incidence of DR. The follow-up period of a year was also relatively short, which limited our ability to detect PDR incidence. We also chose to concatenate DR grades from both eyes to give a more global reflection of a persons' DR status. Although retinal vessel geometry was only assessed in one eye, we believe the associations we found provide further support for the link between the retinal vasculature and a person's overall microvascular health.

In conclusion, our study has shown associations between novel measures of retinal vascular geometry and the incidence and progression of DR. These geometric measures are likely to represent early changes in the retinal microvasculature, and further study is required to determine if they can contribute to the prediction of DR development in a clinical setting.

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References


