Clinical and Epidemiologic Research

Relationship Between Retinal Vascular Geometry With Retinal Nerve Fiber Layer and Ganglion Cell-Inner Plexiform Layer in Nonglaucomatous Eyes

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PURPOSE. To examine the relationship between retinal vascular geometric parameters with retinal nerve fiber layer (RNFL) and ganglion cell-inner plexiform layer (GC-IPL) parameters in nonglaucomatous subjects.

METHODS. Study subjects were identified from the Singapore Chinese Eye Study (SCES), a population-based survey of Singaporean Chinese aged 40 to 80 years. All subjects underwent standardized systemic and ocular examinations. Nonglaucomatous eyes were defined as eyes with normal, reliable visual field results. Retinal vascular parameters (retinal vascular fractal dimension, tortuosity, and caliber) were measured from retinal photographs by using a computer-assisted program, according to a standardized protocol. Spectral-domain optical coherence tomography (SD-OCT) was used to measure RNFL and macular GC-IPL thicknesses.

RESULTS. A total of 352 nonglaucomatous subjects with gradable retinal photographs and OCT images were included for the final analyses. In multiple regression analyses, after adjusting for age, sex, hypertension, diabetes, axial length, disc area, and OCT signal strength; decreased retinal vascular fractal dimension ($\beta = -1.60, P = 0.002$), narrower retinal arteriolar caliber ($\beta = -1.60, P = 0.001$), and venular caliber ($\beta = -1.97, P < 0.001$) were independently associated with thinner average RNFL thickness. In addition, decreased retinal vascular fractal dimension ($\beta = -0.77, P = 0.017$) and decreased retinal venular tortuosity ($\beta = -0.63, P = 0.042$) were independently associated with thinner average GC-IPL thickness after adjusting for age, sex, hypertension, diabetes, axial length, and OCT signal strength.

CONCLUSIONS. Rarefaction, vasoconstriction, and straightening of the retinal vasculature are associated with thinner RNFL and GC-IPL thickness. This information may potentially provide further insights on the role of vascular processes in glaucoma development and progression.

Keywords: retinal vasculature, retinal nerve fiber layer, ganglion cell-inner plexiform layer, optical coherence tomography

Glaucome is a multifactorial optic neuropathy characterized by progressive degeneration of retinal ganglion cells. There is good evidence that vascular risk factors play important roles in the development of glaucoma.1,2 For example, hypertension, nocturnal arterial hypotension, atherosclerosis, and reduced ocular blood flow have been reported as potential risk factors for glaucoma.3–5

Examination of the retinal vascular parameters has provided a useful model to study the role of vascular processes in the development and progression of glaucoma. In this regard, recent population-based studies have demonstrated that narrower retinal vascular caliber, measured by computer-assisted methods, is associated with glaucomatous optic neuropathy, thinner retinal nerve fiber layer (RNFL) thickness, and a thinner neuroretinal rim.6–9 Apart from retinal vascular caliber measurement, newer retinal vascular parameters such as fractal dimension and tortuosity have recently been introduced.10,11 These new retinal vascular parameters provide global quantifications of retinal vasculature that are indicative of the “optimality state” of the retinal vascular network.11,12 These newer retinal vascular parameters have also been linked with diabetic retinopathy and systemic vascular disorders such as hypertension, diabetes, and stroke.13–16

Quantitative measurement of ganglion cell-inner plexiform layer (GC-IPL) thickness with spectral-domain optical coherence tomography (SD-OCT) is now available.17,18 This new OCT parameter has comparable diagnostic performance as conventional peripapillary RNFL thickness and optic nerve parameters.19 Although some previous studies have established the link between retinal vascular caliber and RNFL thickness,7,9 a further comprehensive evaluation involving newer retinal vascular measures reflecting the optimality state of the vascular.
network and macular GC-IPL thickness has not yet been reported. Understanding these relationships may provide additional insights on the influence of retinal vascular architecture on retinal ganglion cell morphology as structural ganglion cell damage often precedes detectable visual function loss in glaucoma.20,21

The aim of this study was to investigate the relationship between a spectrum of retinal vascular parameters with RNFL and macular GC-IPL parameters in a cohort of nonglaucoma-tous patients adults. Information from this study may corroborate the normal anatomic relationship between retinal vascular changes and retinal ganglion cell changes, improve our understanding of the physiological variations of ganglion cell-related structures, and aid in the differentiation between physiological and pathological structural changes related to glaucoma.

METHODS

Study Population

The data for this study were derived from the Singapore Chinese Eye Study (SCES), a population-based cross-sectional study of eye diseases in Chinese adults residing in Singapore, aged between 40 and 80 years.22 The study adhered to the tenets of the Declaration of Helsinki, and ethics committee approval was obtained from the Institutional Review Board of the Singapore Eye Research Institute. Written informed consent was obtained from all participants. All participants underwent standardized and comprehensive systemic and ocular examinations.

Study Subjects

The methodology of the SCES has been described in detail elsewhere.22 In this substudy (conducted between June 2009 and June 2011), subjects who had undergone retinal photography, SD-OCT imaging, and visual field examinations were included. Exclusion criteria included logarithmic minimum angle of resolution (logMAR) >0.5, evidence of maculopathy or retinopathy, previous ocular surgery (except for cataract surgery), neurological diseases or clinical features compatible with a diagnosis of glaucoma suspect, or manifest glaucoma and SD-OCT imaging with signal strength <6. Glaucoma suspect was defined as having any of the following criteria in the presence of normal visual field: intraocular pressure (IOP) >21 mm Hg; signs consistent with pseudoexfoliation or pigment dispersion syndrome; narrow angles (posterior trabecular meshwork visible for <180° during static gonioscopy); and peripheral anterior synechiae or other findings consistent with secondary glaucoma. Glaucoma was defined based on the presence of visual field defects (as described below) regardless of structural features (optic disc or RNFL appearance) to avoid potential bias in the evaluation of RNFL and GC-IPL parameters.

Visual Field Examination

Standardized visual field testing was performed with static automated perimetry (Swedish Interactive Threshold Algorithm standard 24-2, Humphrey Field Analyzer II; Carl Zeiss Meditec, Dublin, CA). A visual field was defined as reliable when fixation losses were less than 20%, and false-positive, false-negative rates were less than 33%. A glaucomatous visual field defect was defined as the presence of three or more significant (P < 0.05) nonedge contiguous points with at least one at the P < 0.01 level on the same side of the horizontal meridian in the pattern deviation plot, and classified as “outside normal limits” on the Glaucoma Hemifield Test, confirmed on two consecutive visual field examinations. All subjects included for the final analysis had reliable, normal visual field.

Retinal Photography and Measurements of Retinal Microvasculature

Digital fundus photography was performed using a 45° digital retinal camera (Canon CR DGi with a SLR digital camera back; Canon Tokyo, Japan) after pupil dilation with tropicamide 1% and phenylephrine hydrochloride 2.5%. Two retinal images
were obtained for each eye, one centered at the optic disc and the other centered at the macula. We used the photographs of the right eye of each participant; if the right eye photograph was ungradable, measurements were performed for the left eye.

We used a semiautomated computer-assisted program (Singapore I Vessel Assessment [SIVA], version 3.0; National University of Singapore, Singapore) to quantitatively measure a range of retinal vascular parameters from digital photographs; namely retinal vascular fractal dimension ($D_f$), tortuosity, and caliber. Trained graders, masked to subjects’ characteristics used the programs to measure the vascular parameters according to a standard protocol. In brief, the computer-assisted programs automatically identified the optic disc with reference to the optic disc center and automatically traced the retinal vessels. To further ascertain the accuracy of the automated vessel tracing generated by the programs, trained graders examined the automatically traced vessels and further performed manual amendments whenever necessary. Details of the standardized grading protocol have been described previously elsewhere.23,26

$D_f$ is a mathematical measure that quantifies complex geometric patterns in objects that are self-similar in their scaling patterns.24 With respect to this, retinal vascular $D_f$ represents a “global” measure of the retinal vascular network complexity that summarizes the branching pattern of the retinal vasculature.10,11 $D_f$ was calculated from skeletonized vessel tracing algorithm using the box-counting method.11 $D_f$ values were measured from optic disc-centered images, and defined as the region from 0.5 to 2.0 optic disc diameters away from the disc margin (Fig. 1). Higher values of $D_f$ indicate a more complex vascular branching pattern and lower $D_f$ values indicate sparser vascular network.

Retinal vascular tortuosity reflects the straightness/curliness of the vessels. Retinal vascular tortuosity was computed as the integral of the curvature square along the path of the vessel, normalized by the total path length; this measure is dimensionless, representing a ratio measure.25 The measurement area of vascular tortuosity was defined as the region from 0.5 to 2.0 optic disc diameters away from the disc margin. The estimates were summarized as retinal arteriolar and venular tortuosity, representing the average tortuosity of arterioles and venules respectively. In general, a smaller tortuosity value indicates a straighter retinal vessel.

Retinal vascular caliber was measured according to the standardized protocol used in the Atherosclerosis Risk in Communities Study (ARIC).23,25 Similarly, the measurement area was defined as the region from 0.5 to 2.0 optic disc diameters away from the disc margin (Fig. 1). Based on the revised Knudston-Parr-Hubbard formula, the retinal arteriolar and venular calibers were summarized as central retinal artery equivalent (CRAE) and central retinal vein equivalent (CRVE), respectively.27 A previous study evaluated the reliability of retinal vascular fractal dimension, tortuosity, and caliber—and reported moderate to high intra- and intergrader reliability.10

**OCT Imaging**

Imaging was performed using HD-OCT equipment (Cirrus HD-OCT; Carl Zeiss Meditec, Dublin, CA) after pupil dilation. Cirrus HD-OCT was used to acquire 1 macular scan and 1 optic disc scan using the macular cube 200 x 200 and optic disc cube 200 x 200 scan protocols, respectively, in each study eye. Details of the HD-OCT (Carl Zeiss Meditec) macular and optic disc scan protocols have been described in detail elsewhere.28 The RNFL algorithm native to Cirrus HD-OCT were used to measure peripapillary RNFL thicknesses (average and quadrants) automatically. Detailed descriptions of the RNFL algorithm has been previously described in detail.29

The ganglion cell analysis (GCA) algorithm incorporated in the HD-OCT software (version 6; Carl Zeiss Meditec) was used to process and measure the macular GC-IPL thickness within a 14.13 mm² elliptical annulus area centered on the fovea. The GCA algorithm automatically segmented the GC-IPL based on three-dimensional data generated from the macular cube 200 x 200 scan protocol. The algorithm automatically segmented the outer boundary of RNFL and the outer boundary of IPL at the macular region; the segmented layer yielded the combined thickness of the GC and IPL. The average and sectoral (supero-temporal, superior, supero-nasal, infero-nasal, inferior, infero-temporal) GC-IPL thicknesses were measured from the elliptical annulus centered on the fovea (Fig. 2). A detailed description of the GCA algorithm has been reported elsewhere.18,30

Subjects with OCT scans showing retinal layer segmentation failure, signal strength <6, or artifacts due to eye movements or blinking were further excluded from the analysis.

**Measurement of Ocular Factors**

All subjects underwent an ophthalmic examination including measurement of logMAR best-corrected visual acuity testing, refraction, axial length (AL) measurement, IOP measurement, gonioscopy, visual field, and fundus examination. IOP was measured with a Goldmann applanation tonometer (GAT; Haag-Streit, Bern, Switzerland) before pupil dilation. The static refraction of each eye was measured using an autorefractor (Canon RK 5 Auto Ref-Keratometer; Canon, Inc., Ltd., Tochigiken, Japan). Spherical equivalent refraction was calcul-
Baseline characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean</th>
<th>SD</th>
<th>Interquartile Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>53.61</td>
<td>6.71</td>
<td>48.04–58.08</td>
</tr>
<tr>
<td>Sex, n (% male)</td>
<td>188 (53.4)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Presence of hypertension, n (%)</td>
<td>126 (35.8)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Presence of diabetes, n (%)</td>
<td>27 (7.9)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>130.26</td>
<td>16.58</td>
<td>119.50–139.50</td>
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<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>77.79</td>
<td>10.00</td>
<td>70.50–83.50</td>
</tr>
<tr>
<td>Blood glucose, mmol/L</td>
<td>6.19</td>
<td>2.43</td>
<td>5.00–6.50</td>
</tr>
<tr>
<td>Glycosylated hemoglobin, %</td>
<td>9.57</td>
<td>0.85</td>
<td>5.60–6.00</td>
</tr>
<tr>
<td>IOP, mm Hg</td>
<td>14.22</td>
<td>2.66</td>
<td>12.00–15.00</td>
</tr>
<tr>
<td>Spherical equivalent, D</td>
<td>–1.12</td>
<td>2.45</td>
<td>–2.37–0.47</td>
</tr>
<tr>
<td>Axial length, mm</td>
<td>24.15</td>
<td>1.19</td>
<td>23.25–24.86</td>
</tr>
<tr>
<td>Presence of cataract, n (%)</td>
<td>45 (12.8)</td>
<td>—</td>
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</table>

Retinal vascular parameters

- Arteriolar tortuosity, × 10⁴: 1.451, 0.049, 1.424–1.483
- Venular tortuosity, × 10⁴: 0.85, 0.21, 0.70–1.00
- Arteriolar caliber, μm: 121.74, 12.99, 111.27–130.48
- Venular caliber, μm: 181.15, 20.04, 167.15–191.52

OCT parameters

- Average RNFL thickness, μm: 96.57, 8.98, 90.89–102.54
- Superior RNFL thickness, μm: 119.84, 15.92, 110.03–129.96
- Inferior RNFL thickness, μm: 125.13, 16.62, 114.80–136.14
- Average GC-IPL thickness, μm: 82.75, 5.65, 79.00–86.00
- Superior GC-IPL thickness, μm: 83.31, 5.73, 81.00–89.75
- Inferior GC-IPL thickness, μm: 80.17, 5.98, 77.00–84.00
- Inferior nasal GC-IPL thickness, μm: 83.24, 6.30, 79.00–87.00
- Superior temporal GC-IPL thickness, μm: 85.42, 6.31, 81.00–89.75
- Inferior temporal GC-IPL thickness, μm: 82.54, 5.75, 79.00–86.00

OCT analysis

- Presence of cataract: 45 (12.8)
- Axial length, mm: 24.15 ± 1.19

Measurement of Systemic Factors

Systolic and diastolic blood pressures were measured using a digital automatic blood pressure monitor (Dinamap model Pro Series DP110X-RW, 100V2; GE Medical Systems Information Technologies, Inc., Milwaukee, WI), after subjects were seated for at least 5 minutes. Two measurements were obtained, 5 minutes apart. A third measurement was made if the systolic pressure readings differed by more than 10 mm Hg or the diastolic pressure readings by 5 mm Hg. The mean of the two closest readings was then recorded as the subject’s blood pressure. Hypertension was defined as systolic blood pressure of ≥140 mm Hg or diastolic pressure of ≥90 mm Hg at the time of the examination, or a reported history of physician-diagnosed hypertension or self-reported history of antihypertensive medication use, or both.

Nonfasting venous blood samples were analyzed at the National University Hospital Reference Laboratory for biochemistry and enzymology testing of glycated hemoglobin (HbA1c) and serum glucose level. Diabetes mellitus was defined as nonfasting glucose ≥11.1 mmol/L or subjects’ self-reported use of diabetic medication or a reported history of physician-diagnosed diabetes.

Statistical Analysis

All statistical analyses were performed using statistical software (SPSS version 17.0; SPSS, Inc., Chicago, IL). The normality of data distribution was assessed by skewness test, kurtosis test, and Shapiro-Wilk (SW) test. The mean, standard deviation, and interquartile range were calculated for demographic, baseline characteristics, retinal vascular parameters, RNFL, and macular GC-IPL thickness. Multiple linear regression models were used to estimate the change in RNFL/GC-IPL thickness (dependent variables) for each standard deviation change in retinal vascular parameters (independent variables). The analysis was adjusted for age, sex, hypertension, diabetes, axial length, disc area, and presence of macular or vitreoretinal diseases (n = 10). Hence, a total of 352 nonglaucomatous subjects were included for the final analysis. Table 1 shows the demographic characteristics of study subjects included for analyses (n = 352).
Table 2 shows the relationships between retinal vascular parameters and RNFL thickness parameters. $D_f$ retinal arteriolar, and venular caliber were significantly associated with average, superior, and inferior RNFL thicknesses after adjustment for age and sex. In the multivariable analyses, these associations remained consistent after further adjustment for age, sex, hypertension, diabetes, axial length, disc area, and OCT signal strength. Inclusion of IOP as a covariate in the multivariable model did not change the trend and magnitude of findings significantly (data not shown). Each SD decrease in $D_f$ was associated with a 1.60-μm decrease in average RNFL thickness ($P = 0.002$). In addition, each SD decrease in retinal arteriolar caliber was associated with a 1.60-μm decrease in average RNFL thickness ($P = 0.001$). Each SD decrease in retinal venular caliber was associated with a 1.97-μm decrease in average RNFL thickness ($P < 0.001$).

Table 3 shows the relationships between retinal vascular parameters and GC-IPL thickness parameters. $D_f$ retinal venular tortuosity, retinal arteriolar, and venular caliber were significantly associated with average, superior, and inferior (except for venular tortuosity) GC-IPL thicknesses after adjustment for age and sex. Nevertheless, after further adjustment for age, sex, hypertension, diabetes, axial length, and OCT signal strength in the multivariable analyses, only $D_f$ and retinal venular tortuosity were significantly associated with average, superior, and inferior (except for venular tortuosity) GC-IPL thicknesses. Similar trends of associations were observed between retinal vascular parameters and GC-IPL thicknesses in the superior temporal, superior nasal, inferior temporal, and inferior nasal sectors (data not shown). Each SD decrease in $D_f$ was associated with a 0.77-μm decrease in average GC-IPL thickness ($P = 0.017$). In addition, each SD decrease in retinal venular tortuosity was associated with a 0.63-μm decrease in average GC-IPL thickness ($P = 0.042$).

**Discussion**

Detailed understanding of the relationship between retinal vasculature and optic nerve is essential to further elucidate the role of vascular process in glaucoma development and progression. In this study among nonglaucomatous eyes, we showed that sparser retinal vasculature (decreased $D_f$) was associated with thinner RNFL and GC-IPL thicknesses. In addition, straighter retinal vessels were associated with thinner GC-IPL thicknesses. These findings may improve our understanding on the influence of retinal vascular architecture on retinal ganglion cell morphology. We previously reported in healthy Asian adults and children that narrower retinal vascular caliber and straighter vessels were associated with thinner RNFL and neuroretinal rim area, measured by time-domain OCT and scanning laser ophthalmoscopy respectively. To date, however, no studies have explored the relationship between retinal vascular parameters and macular GC-IPL thickness, which is a new spectral domain-OCT parameter. Evaluation of GC-IPL is of particular interest as GC-IPL thinning may represent early structural changes in glaucoma. To our knowledge, this is the first study to explore detailed relationships between retinal vascular geometry and RNFL and GC-IPL parameters. These new retinal vascular parameters, together with conventional retinal vascular caliber measurements, may more comprehensively indicate the optimality of the retinal microcirculation, and provide a broader frame-
### TABLE 3. Relationship between Retinal Vascular Geometric Parameters and Macular GC-IPL Thickness Parameters

<table>
<thead>
<tr>
<th>Average GC-IPL Thickness, µm Superior GC-IPL Thickness, µm</th>
<th>Inferior GC-IPL Thickness, µm</th>
<th>Adjusted for age, sex, hypertension, diabetes, Al, and OCT signal strength.</th>
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<tr>
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<tr>
<td><strong>Age, Sex-Adjusted</strong></td>
<td><strong>Multivariable-Adjusted</strong></td>
<td><strong>Multivariable-Adjusted</strong></td>
</tr>
<tr>
<td><strong>Mean (95% CI)</strong></td>
<td><strong>Mean (95% CI)</strong></td>
<td><strong>Mean (95% CI)</strong></td>
</tr>
<tr>
<td>arteriolar tortuosity, 10⁻⁴</td>
<td>0.32 (0.19 to 0.46)</td>
<td>0.34 (0.20 to 0.48)</td>
</tr>
<tr>
<td>arteriolar caliber</td>
<td>0.94 (1.53 to 0.30)</td>
<td>0.92 (1.46 to 0.29)</td>
</tr>
<tr>
<td>venular tortuosity, 10⁻⁴</td>
<td>0.68 (1.02 to 0.21)</td>
<td>0.72 (1.39 to 0.25)</td>
</tr>
<tr>
<td>venular caliber</td>
<td>0.94 (1.53 to 0.30)</td>
<td>0.92 (1.46 to 0.29)</td>
</tr>
<tr>
<td>arteriolar caliber</td>
<td>0.94 (1.53 to 0.30)</td>
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<tr>
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</tr>
<tr>
<td>venular caliber</td>
<td>0.94 (1.53 to 0.30)</td>
<td>0.92 (1.46 to 0.29)</td>
</tr>
</tbody>
</table>

**P < 0.05.**

**Denotes P < 0.001.**

**Adjusted for age, sex, hypertension, diabetes, Al, and OCT signal strength.**

In our study, we also observed that narrower retinal vascular caliber were associated with thinner RNFL thickness. This coincided with our previous findings which reported similar findings in healthy Asian adults and children. Furthermore, previous population-based studies reported that thinner retinal vascular caliber was associated with both prevalent and incident glaucoma. These findings may be explained by the hypothesis that thinner RNFL may have lower metabolic levels and thus lower vascular demand, leading to thinner vascular caliber. In addition, it was also postulated that narrower venular caliber may indicate venous congestion, which may result in cytotoxic and vasogenic edema. These effects of venous congestion may in turn lead to secondary constriction of arterioles and further vascular dysregulation via venoarteriolar response. These sequential events of vascular dysregulation may potentially lead to early RNFL thinning.

Vascular tortuosity is largely dependent on the properties of the endothelium, which lines the inner vessel wall. This is because the endothelium autoregulates tissue perfusion by secreting chemical mediators such as nitric oxide and endothelin. These chemicals are thought to stimulate angiogenesis, which subsequently increases vascular tortuosity and thus enhances tissue perfusion and nourishment of relevant tissue area. This postulation was reflected in our observations where decreased retinal venular tortuosity (straighter venules) was associated with thinner GC-IPL thickness. This suggests that decreased vascular tortuosity may possibly lead to degenerative ganglion cell changes because of poorer tissue nourishment. Our previous study work on the link between retinal microcirculation and glaucoma-related structural changes.

An optimal retinal vasculature as a fractal is associated with greater efficiency in blood circulation with lower energy utilization. In this study, we observed that decreased retinal vascular fractal dimension was associated with thinner RNFL, GC-IPL thicknesses, which are indicative of glaucomatous structural changes. Figure 1 further illustrates that eyes with sparser and narrower retinal vasculature are coupled with thinner RNFL and GC-IPL thicknesses and vice versa. Our findings may lend further support to the postulation that ocular vascular regulatory disruption is linked with changes in retinal ganglion cell structures. Our previous findings from the Singapore Malay Eye Study also revealed that decreased retinal vascular fractal dimension was associated with ocular hypertension, which is a strong predictor factor of glaucoma. Taken together, these findings warrant further investigations, particularly prospective ones, to confirm the influence of retinal vascular fractal dimension on glaucoma.
also similarly reported that straighter retinal vessels were associated with a thinner neuroretinal rim area, measured by scanning laser ophthalmoscopy.\textsuperscript{33} In addition, Wu et al. reported in the Singapore Malay Eye Study that decreased retinal venular tortuosity was associated with primary open angle glaucoma.\textsuperscript{34} These findings collectively indicate that decreased vascular tortuosity may be linked with tissue perfusion impairment, thus potentially leading to early GC-IPL thinning. Further replication of our findings in other populations may help to confirm this exploratory finding.

The strengths of our study include a large sample size with a single common ethnicity. Hence, our findings were unlikely to be confounded by ethnic heterogeneity. In addition, standardized clinical examination protocols, reliable quantifications of retinal vascular parameters, and OCT parameters were used in our study. Nevertheless, this study has a few limitations. First, the causative relationship between retinal vascular geometry and RNFL/GC-IPL thickness cannot be definitively assessed due to the cross-sectional nature of our data. Nonetheless, our findings warrant further longitudinal evaluations of the causal link between retinal microvascular changes and glaucomatous structural changes. Second, despite adjustments for diabetes status, we may not entirely exclude the possibility that our findings were partly confounded by the effect of tissue hypoxia related to diabetes. Nevertheless, in a supplementary analysis including only systemically healthy subjects (without hypertension and diabetes, n = 213; data not shown), the trends of associations between retinal vascular parameters with RNFL and GC-IPL parameters remained the same. Third, our study was a subsample selected from a population-based study (SCES). The SCES subjects included in this analysis were significantly younger, less likely to have hypertension, diabetes, and cataract compared with those excluded from this subsample (data not shown). Thus, our study sample may be subjected to selection biases and this may limit the generalizability of our results. Fourth, despite the standardized protocols used, the precision of retinal vasculature grading may be affected by measurement errors related to subjective grader input (both intra- and intergrader variability), variability in image contrast or brightness, and other unknown factors (e.g., pulse cycle).\textsuperscript{36,37} These factors could have biased or modified the associations observed in our sample. Since this is the first study to evaluate the relationships between retinal vascular geometry and RNFL and GC-IPL parameters, some findings may be viewed as exploratory and further confirmatory studies are necessary.

In conclusion, we found that retinal vasculature were sparser, narrower, and straighter among eyes with thinner RNFL and GC-IPL thicknesses. Our study further demonstrates the intimate link between retinal microvascular changes and glaucoma-related structural changes. This information may potentially provide further insights on the role of vascular processes in the development of glaucoma and its progression.

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