Glaucoma is a leading cause of global irreversible blindness and affects 64.3 million individuals worldwide. To date, the actual pathogenesis of glaucoma and primary open-angle glaucoma (POAG) is not entirely understood. There is good evidence that vascular factors play important roles in the development of glaucoma. For example, hypertension, nocturnal hypotension, and reduced ocular perfusion may affect ocular microcirculation and blood supply to the optic nerve head, thus leading to glaucoma. Structural changes seen in the retinal vessels, presumably reflecting alterations in the circulation system, have also been linked to glaucoma, and thus, examination of the retinal vascular caliber may provide a useful model with which to evaluate the role of vascular processes in the development of glaucoma. In this regard, there have been previous cross-sectional studies that have shown narrower retinal vascular calibers were associated with prevalent glaucoma. In addition, a 10-year prospective population-based study further demonstrated that baseline retinal arteriolar narrowing was associated with incident POAG. This finding may further support the notion of a causal association between vascular processes that are seen in the retinal microvasculature and development of glaucoma. Nevertheless, these previous findings still do not inform us about the specific mechanistic relationship between retinal vascular narrowing and development of glaucoma.

Furthermore, previous studies in children and nonglaucomatous subjects have reported that retinal vascular narrowing was associated with loss of optic nerve tissue and axons, which were manifested in the form of neuroretinal rim loss and thinning of peripapillary retinal nerve fiber layer (RNFL) thickness. Taken together, these previous findings appear to be consistent with the vascular theory of glaucoma pathogenesis which postulates that impairment of the microvasculature network at the retinal and optic nerve head (ONH)
retinal ganglion cell axons. These ischemia-related events may then lead to retinal ganglion cell degeneration, thus initiating glaucomatous damage. Nevertheless, holistic evaluation of the interrelationships among retinal vascular narrowing, RNFL thinning, and glaucoma has not been performed in a single study. In this aspect, the mediation framework approach may be used to further test the vascular theory.

Mediation refers to the “transmission” of the effect of an explanatory variable on an outcome variable through an intermediate variable. This intermediate variable is termed the mediator. Mediation effect is also referred to as the indirect effect. The magnitude of the indirect effect indicates the amount of “transmission” through the relevant mediator variable. Such a model hypothesizes that an exposure influences a mediator which then affects the outcome. Thus, the mediation model may potentially explicate the mechanism that underlies an observed association between an exposure and outcome via the inclusion of a mediating factor. This approach may potentially allow us to validate the assumption of whether the association between retinal vascular narrowing with glaucoma operates through RNFL thinning and, if so, the extent of this effect via RNFL thinning.

The aim of this study was to explore the interrelationships among retinal vascular caliber, RNFL, and glaucoma; specifically, whether the association between retinal vascular narrowing and glaucoma is “mediated” through RNFL thinning, as postulated by the vascular theory, using the mediation analysis approach. In contrast to previous studies, which only evaluated the associations between retinal vascular calibers and glaucoma,8–10,22,23 findings from this current study could potentially provide deeper insights into the mechanistic pathway(s) between retinal vascular narrowing and glaucoma.

**METHODS**

**Study Populations**

The Singapore Epidemiology Eye Disease (SEED) study is a population-based cross sectional study consisting of three major ethnic groups in Singapore: Malays (the Singapore Malay Eye Study, 2004–2006), Indians (the Singapore Indian Eye Study, 2007–2009), and Chinese (the Singapore Chinese Eye Study, 2009–2011). Details of the study design and methodology of the SEED study have been reported elsewhere. Briefly, the study was conducted in the southwestern part of Singapore, using a standardized study protocol across the three ethnic groups of subjects. Age-stratified random sampling strategy was adopted in each ethnic group to select adults between 40 and 80 years of age. Overall, a total of 4168 Malays, 4497 Indians, and 4605 Chinese were identified and invited to participate in the study. A total of 10,333 subjects participated between 40 and 80 years of age. Overall, a total of 4168 Malays, 4497 Indians, and 4605 Chinese were identified and invited to participate in the study. A total of 10,333 subjects participated.

**Clinical Ocular Examinations**

All subjects underwent a standardized interview and comprehensive ocular examinations at the Singapore Eye Research Institute. Briefly, intraocular pressure (IOP) was measured using the Goldmann applanation tonometer (Haag-Streit, Bern, Switzerland) before pupil dilation. One reading was taken from each eye. If the IOP reading was greater than 21 mm Hg, a repeat reading was taken, and the second reading was used for analysis. Refractive error and corneal curvature were measured using an autorefractor (RK-5 model autorefractor keratometer; Canon, Inc., Tokyo, Japan); and the mean of five measurements was used for analysis. Spherical equivalent was calculated as the spherical value plus half of the negative cylinder value. Axial length was measured using noncontact partial coherence interferometry (intraocular lens [IOL] Master version 3.01; Carl Zeiss Meditec AG, Jena, Germany); and the means of five measurements were used for analysis. Gonioscopy was performed using a Goldmann two-mirror lens (Ocular Instruments, Inc., Bellevue, WA, USA) under standard dark illumination in patients suspected of having glaucoma (as defined below), subjects with temporal peripheral Van Herick grade 2 or less, and 1 in 5 randomly selected participants who did not meet the first 2 criteria.

After pupil dilation, the optic disc was evaluated using a +78 diopter (D) lens at 10X magnification with a measuring graticule (Haag-Streit) during slit lamp funduscopy (model BQ-900; Haag-Streit). The clinical vertical cup-to-disc ratio (VCDR) was calculated accordingly, and morphological features such as disc hemorrhage, notching, and defects of the RNFL were documented.

**Visual Field Examinations**

Static automated perimetry (Swedish interactive threshold algorithm standard 24-2; Humphrey field analyzer II; Carl Zeiss Meditec) was performed in one in five participants and in all suspected of having glaucoma (as defined below). A visual field was defined as reliable when fixation losses were less than 20%, and false positive and false negative rates were less than 35%. Visual field test was repeated if the test result was unreliable. A glaucomatous visual field defect was defined as the presence of 3 or more significant (P < 0.05) non–edge-contiguous points with at least 1 point at a P level of <0.01 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.

**Retinal Photography and Measurements of Retinal Microvasculature**

Digital fundus photography was performed using a 45° digital retinal camera (CR DGi model with a single-lens reflex digital camera back; Canon) after pupil dilation with tropicamide 1% and phenylephrine hydrochloride 2.5%. An optic disc-centered fundus photograph was obtained for each eye.

We used a semiautomated computer-assisted program (Integrative Vessel Analysis [IVAN], University of Wisconsin, Madison, WI, USA), according to a standardized protocol, which has been described previously. Briefly, all vessels coursing through a specified area of 0.5 to 1.0 disc diameter from the disc margin were automatically traced and identified as arterioles or venules, respectively. A trained grader masked to participants’ characteristics further performed manual amendments whenever necessary. Based on the revised Knudtson-Parr-Hubbard formula, the measurements of retinal arteriolar and venular calibers were summarized as central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE), respectively.

**ONH Imaging and RNFL Measurement**

ONH imaging was performed using Heidelberg retinal tomography (HRT; Tomograph II; Heidelberg Engineering, GmbH, Dossenheim, Germany), a confocal scanning ophthalmoscope,
after pupil dilation. HRT II cylindrical corrective lenses were used for subjects with astigmatism \( \geq 1.0 \) D. Imaging was performed after entering subject’s corneal curvature data to correct for ocular magnification. After the images were acquired, the disc margin was manually demarcated and defined as the inner edge of Elschnig’s ring. The system’s built-in software then provided an indirect measurement of the global mean RNFL thickness, which was defined as the mean surface height of the 360° disc contour line from a standard reference plane, which was defined as 50 μm posterior to the mean retinal surface in the region of 350° to 356° along the drawn disc margin contour. Each image was coupled with a standard deviation (SD) value to reflect the image quality; scans with SD greater than 50 μm were excluded.

**Other Measurements**

A detailed interviewer-administered questionnaire was used to collect demographic data, medical history, and ocular history. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using a digital, automatic blood pressure monitor (Dinamap model Pro series DP110X-RW, 100V2; GE Medical Systems Information Technologies, Inc., Milwaukee, WI, USA), after subjects were seated for at least 5 minutes. Blood pressure measurements were made twice, 5 minutes apart. A third measurement was made if the previous two SBP readings differed by more than 10 mm Hg or the DBP by more than 5 mm Hg. The mean between the closest two blood pressure readings was then taken. Mean arterial BP (MAP) was calculated as \([DBP + 1/3(SBP - DBP)]\). Ocular perfusion pressure (OPP) was defined as \([2/3(MAP - IOP)]\). Hypertension was defined as SBP \( \geq 140 \) mm Hg or DBP \( \geq 90 \) mm Hg, self-reported history of physician-diagnosed hypertension, or use of antihypertensive medication. Diabetes was defined as a nonfasting glucose level \( \geq 11.1 \) mmol/L, use of diabetic medication, or self-reported history of diabetes.

**Glaucoma Diagnostic Definitions**

A patient with suspected glaucoma was defined as having any of the following criteria: (1) IOP \( > 21 \) mm Hg; (2) VCDR \( > 0.6 \) or VCDR asymmetry \( > 0.2 \); (3) signs consistent with pseudoxefoliation or pigment dispersion syndrome; (4) narrow angles (posterior trabecular meshwork visible for \( < 180° \) during static gonioscopy); (5) peripheral anterior synchiae; (6) other findings consistent with secondary glaucoma; and (7) known history of glaucoma.

Glaucoma was defined according to International Society of Geographical and Epidemiological Ophthalmology criteria based on three categories.31 Category 1 cases were defined as having optic disc abnormality (VCDR or VCDR asymmetry \( \geq 97.5 \)th percentile or neuroretinal rim width between position 11- and 1-o’clock or 5- and 7-o’clock \( < 0.1 \) VCDR), with a corresponding glaucomatous visual field defect. Category 2 cases were defined as having a severely damaged optic disc (VCDR or VCDR asymmetry \( \geq 99.5 \)th percentile) in the absence of adequate performance in a visual field test. Category 3 cases were defined as subjects without visual field or optic disc data who were blind (corrected visual acuity of \( < 3/60 \)) and who had previous glaucoma surgery or had IOP \( > 99.5 \)th percentile.

A narrow anterior chamber angle was diagnosed if the posterior trabecular meshwork was seen for \( 180° \) or less of the angle circumference during static gonioscopy. Primary angle closure glaucoma (PACG) was defined as an eye with glaucoma accompanied by the presence of narrow anterior chamber angle and features of trabecular obstruction by peripheral iris (such as peripheral anterior synchiae, elevated IOP, iris whirling, “glaukomflecken” lens opacities, or excessive pigment deposition on the trabecular surface). Subjects with glaucoma and an open, normal drainage angle with no identifiable secondary pathologic processes were defined as having POAG.

**Statistical Analysis**

In our analyses, retinal vascular calibers were treated as the exposure (denoted \( X \)), mean RNFL thickness was treated as the mediating factor (denoted \( M \)), and glaucoma was the outcome (denoted \( Y \)).

In the first stage of analysis, logistic regression analysis was performed to evaluate relationship between retinal vascular calibers and glaucoma after adjusting for relevant covariates. In mediation terminology, the effect estimates of retinal vascular calibers obtained in these logistic regression models were defined as the “total effects” (coefficient \( c \)) of retinal vascular calibers on glaucoma, which did not include the mediating factor \( M \) in the models (Equation 1).

\[
Y = cX + E_1,
\]

where \( E_1 \) refers to error term.

In the second stage of analysis, direct effects of retinal vascular calibers on glaucoma were determined. Direct effect (coefficient \( c' \)) refers to the effect estimate of retinal vascular calibers on glaucoma after controlling for RNFL thickness (mediating factor, \( M \)), as specified in Equation 2.

\[
Y = c'X + bM + E_2,
\]

where \( E_2 \) refers to error term.

The effect estimates of RNFL thickness on glaucoma is denoted coefficient \( b \) (Equation 2). Generally, substantial effect size reduction in direct effect coefficient, \( c' \), from the total effect coefficient, \( c \), is suggestive of the presence of mediation effect (also known as indirect effect).18

In addition, the effect estimates of retinal vascular calibers on RNFL thickness (mediating factor) is denoted coefficient \( a \), as specified in Equation 3. The coefficient \( a \) was estimated by linear regression.

\[
M = aX + E_3,
\]

where \( E_3 \) refers to an error term.

In the third stage of analysis, regression-based mediation analysis was performed to evaluate the effects of CRAE and CRVE on glaucoma mediated by RNFL thickness. Such effects are known as the indirect effects. Briefly, we computed indirect effects by using the product-of-coefficients approach,19 where coefficients \( a \) and \( b \) were restandardized and multiplied. The purpose of restandardization was to make the coefficients of \( a \) and \( b \) comparable, as both coefficients were originally estimated from different equations and different scales of linear regression and logistic regression models, respectively. In the standardization process, each coefficient was multiplied by the SD of the predictor variable and divided by the SD of the outcome variable of respective equation. Details of this standardization process have been described previously.19,32 The regression models of total, direct, and indirect effects in mediation framework are further illustrated in the figure.

Standard errors and 95% confidence intervals (CIs) of the indirect effects were then calculated using bootstrap sampling with 10,000 replications.33,34 The proportion of effect mediated was calculated as the ratio of the indirect effect estimate to the total effect estimate.35 All statistical analyses were performed using Stata version 12.1 software (College Station, TX, USA).
RESULTS

Of the 10,030 study subjects, 455 subjects did not have fundus photos taken, and 168 subjects were excluded because fundus images in both eyes were of poor quality, leaving a total of 9,407 subjects for this analysis. Right eye was selected as the study eye for each subject; in the event that the right eye's fundus image was not available or ungradable, the left eye was selected instead. Generally, included subjects were younger, less likely to have diabetes and hypertension, and were less myopic (Table 1).

Among the included participants, glaucoma (defined as POAG and PACG combined) was present in 253 participants. Of those participants, 195 cases were POAG and 58 cases were PACG. Overall, participants who had glaucoma were older and more likely to be male and hypertensive (Table 2). In addition, participants who had glaucoma also had significantly higher IOP, longer axial length, more negative spherical equivalent, larger VCDR, and thinner mean RNFL thickness (all \( P < 0.05 \)).

Table 3 shows the associations of CRAE and CRVE with glaucoma and its subtype. After we adjusted for age, sex, ethnicity, hypertension, diabetes, body mass index, spherical equivalent, IOP, narrower CRAE (per SD [15.1 \( \mu m \)] decrease) was associated with increased risk of glaucoma (odds ratio [OR], 1.09; \( P = 0.006 \)), and POAG (OR, 1.23; \( P = 0.006 \)), respectively. Similarly, narrower CRVE (per SD [21.6 \( \mu m \)] decrease) was associated with increased risk of glaucoma (OR, 1.51; \( P < 0.001 \)) and POAG (OR, 1.64; \( P < 0.001 \)), respectively. These associations remained largely similar when the models were adjusted for OPP instead of IOP. We did not observe any significant associations between CRAE and CRVE with PACG.

The direct effects of CRAE on glaucoma (OR, 1.09; \( P = 0.272 \)) and POAG (OR, 1.15; \( P = 0.177 \)) were not significant, although adjusted for the same above-mentioned covariates (Table 4). On the other hand, we observed significant indirect effects of CRAE on glaucoma (OR, 1.02; \( P < 0.001 \)) and POAG (OR, 1.03; \( P < 0.001 \)), through thinning of RNFL thickness. The proportion mediated by thinning of RNFL thickness was 36.9% in glaucoma and 32.3% in POAG (Table 4). In CRVE, both the direct (OR, 1.46; \( P < 0.001 \)) and the indirect (OR, 1.03; \( P < 0.001 \)) effects on glaucoma were significant (Table 4). Similarly, both the direct (OR, 1.58; \( P < 0.001 \)) and the indirect (OR, 1.04; \( P < 0.001 \)) effects of CRVE on POAG were also significant (Table 4). The proportion mediated by RNFL thickness was 12.9% in glaucoma and 12.3% in POAG.

DISCUSSION

We report the relationship between retinal vascular caliber and glaucoma in this study of nearly 10,000 Asian participants. Specifically, we investigated the potential mechanistic network linking retinal vascular calibers, RNFL, and glaucoma by using the mediation analysis approach. Our results indicate that the effect of retinal vascular narrowing on glaucoma was partially the result of thinning of RNFL. These findings provide further mechanistic insights into the link between microvascular changes and glaucoma.

We found that narrowing of retinal arterioles and retinal venules were associated with increased risk of glaucoma and POAG. These associations remained even after adjusting for OPP, which is an important determinant of ocular blood flow.\(^\text{17}\) Our findings in a multiethnic Asian population were consistent with those reported in two other Chinese population-based studies,\(^\text{10,23}\) and one Caucasian population-based study.\(^\text{9}\) Nevertheless, both the Rotterdam Study and the Beaver Dam Eye Study of Caucasian populations did not find significant associations between retinal vascular caliber and glaucoma.\(^\text{22,36}\) This suggests that the relationship between retinal...
vascular narrowing and glaucoma may differ with ethnicity, with, potentially, a more pronounced effect in Asians. This warrants further investigations in other population-based studies. Furthermore, as expected, we did not observe significant associations between retinal vascular calibers and PACG. This may be explained by the natural history of PACG, which is mainly attributed to elevation of IOP due to angle closure instead of dysfunctions in ocular circulation.37,38 Hence, the overall association between retinal vascular calibers and glaucoma was mainly attributed to POAG.

The direct effect analyses, which additionally accounted for RNFL thickness, showed that the direct effects of CRAE on glaucoma and POAG were not significant and had smaller magnitude of effects than its total effect measures on glaucoma and POAG, respectively. In addition, the indirect effects of CRAE on glaucoma and POAG via RNFL thinning were significant with substantial proportion mediated (36.9% in glaucoma and 32.3% in POAG). These collectively provide good evidence that the effects of CRAE narrowing on glaucoma and POAG, respectively, and also with a modest mediation effect through RNFL thinning. We also repeated the above-mentioned analyses using structural equation modeling (SEM) function, which is another analytic method for mediation analysis. Using the SEM approach, we observed identical indirect effect estimates for both CRAE and CRVE on glaucoma and POAG, respectively; and also with a P value of <0.001 (data not shown).

Although both CRAE and CRVE had significant indirect effects on glaucoma and POAG, the proportion mediated by RNFL thinning was evidently greater for CRAE than for CRVE. This may be explained by the different involvement of CRAE and CRVE in the pathogenesis of glaucoma, where narrowing of CRAE may impact the development of glaucoma more directly. CRAE is a proxy measurement of the retinal arterioles, which provide blood supply to the inner retina including the RNFL.15 Thus, narrowing of CRAE may directly impact the development of glaucoma, which is characterized by structural ONH damage and visual field loss.2,6,39

On the other hand, in CRVE-related analysis, the direct effects of CRVE on glaucoma and POAG were significant, and the direct effect measures were similar to those of total effects, implying little effect of mediation through RNFL thickness. Although the indirect effects of CRVE on glaucoma and POAG were statistically significant, the proportions mediated were small (12.9% in glaucoma, 12.3% in POAG), further indicating modest mediation effect through RNFL thinning. We also repeated the above-mentioned analyses using structural equation modeling (SEM) function, which is another analytic method for mediation analysis. Using the SEM approach, we observed identical indirect effect estimates for both CRAE and CRVE on glaucoma and POAG, respectively; and also with a P value of <0.001 (data not shown).

Although both CRAE and CRVE had significant indirect effects on glaucoma and POAG, the proportion mediated by RNFL thinning was evidently greater for CRAE than for CRVE. This may be explained by the different involvement of CRAE and CRVE in the pathogenesis of glaucoma, where narrowing of CRAE may impact the development of glaucoma more directly. CRAE is a proxy measurement of the retinal arterioles, which provide blood supply to the inner retina including the RNFL.15 Thus, narrowing of CRAE may directly impact the development of glaucoma, which is characterized by structural ONH damage and visual field loss.2,6,39

### Table 2. General Characteristics of Included Participants

<table>
<thead>
<tr>
<th>Characteristic*</th>
<th>Persons Without Glaucoma, n = 9154</th>
<th>Persons With Glaucoma, n = 253</th>
<th>Mean/Proportion Difference</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>Mean/Number SD/%</td>
<td>Mean/Number SD/%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>58.0/10.0 10.0</td>
<td>64.1/10.4 10.4</td>
<td>6.1</td>
<td>4.8 to 7.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malay</td>
<td>2887/31.6% 31.6%</td>
<td>102/40.3% 40.3%</td>
<td>8.7%</td>
<td>2.6% to 14.8%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Indian</td>
<td>3136/34.3% 34.3%</td>
<td>101/39.9% 39.9%</td>
<td>5.8%</td>
<td>0.5% to 11.9%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Chinese</td>
<td>3131/34.1% 34.1%</td>
<td>101/39.9% 39.9%</td>
<td>5.8%</td>
<td>0.5% to 11.9%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>5485/59.9% 59.9%</td>
<td>101/39.9% 39.9%</td>
<td>5.8%</td>
<td>0.5% to 11.9%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2098/22.9% 22.9%</td>
<td>67/26.5% 26.5%</td>
<td>6.6%</td>
<td>4.7% to 7.6%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.4/4.7</td>
<td>25.1/4.7</td>
<td>0.3</td>
<td>0.0 to 0.6</td>
<td>0.061</td>
</tr>
<tr>
<td>Intraocular pressure, mm Hg</td>
<td>15.1/3.2</td>
<td>17.0/4.8</td>
<td>1.9</td>
<td>1.5 to 2.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ocular perfusion pressure, mm Hg</td>
<td>55.6/8.3</td>
<td>55.9/8.6</td>
<td>0.3</td>
<td>0.7 to 1.3</td>
<td>0.606</td>
</tr>
<tr>
<td>Spherical equivalent, D</td>
<td>-0.26/2.22</td>
<td>-0.54/2.61</td>
<td>0.28</td>
<td>0.00 to 0.56</td>
<td>0.040</td>
</tr>
<tr>
<td>Axial length, mm</td>
<td>23.6/1.2 1.2</td>
<td>23.7/1.3</td>
<td>0.1</td>
<td>0.0 to 0.3</td>
<td>0.108</td>
</tr>
<tr>
<td>Vertical cup-to-disc ratio</td>
<td>0.40/0.12</td>
<td>0.71/0.14</td>
<td>0.31</td>
<td>0.29 to 0.32</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean retinal nerve fiber layer thickness, μm</td>
<td>250.48/77.92</td>
<td>208.47/105.57</td>
<td>42.01</td>
<td>31.35 to 52.67</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

### Table 3. Total Effects of Retinal Vascular Calibers on Glaucoma, Primary Open-Angle Glaucoma, and Primary Angle Closure Glaucoma

<table>
<thead>
<tr>
<th>Model</th>
<th>Glaucoma, n = 253</th>
<th>POAG, n = 195</th>
<th>PACG, n = 58</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRAE, per SD decrease (per 15.1 μm)</td>
<td>OR 95% CI P Value</td>
<td>OR 95% CI P Value</td>
<td>OR 95% CI P Value</td>
</tr>
<tr>
<td>1*</td>
<td>1.21 1.06–1.38 0.006</td>
<td>1.23 1.06–1.43 0.006</td>
<td>1.14 0.86–1.51 0.361</td>
</tr>
<tr>
<td>2†</td>
<td>1.22 1.06–1.39 0.004</td>
<td>1.25 1.07–1.45 0.004</td>
<td>1.11 0.84–1.48 0.461</td>
</tr>
<tr>
<td>CRVE, per SD decrease (per 21.6 μm)</td>
<td>OR 95% CI P Value</td>
<td>OR 95% CI P Value</td>
<td>OR 95% CI P Value</td>
</tr>
<tr>
<td>1*</td>
<td>1.51 1.32–1.73 &lt;0.001</td>
<td>1.64 1.41–1.91 &lt;0.001</td>
<td>1.15 0.86–1.53 0.341</td>
</tr>
<tr>
<td>2†</td>
<td>1.52 1.32–1.74 &lt;0.001</td>
<td>1.65 1.41–1.93 &lt;0.001</td>
<td>1.11 0.83–1.47 0.483</td>
</tr>
</tbody>
</table>

CI, confidence interval; OR, odds ratio; PACG, primary angle closure glaucoma; POAG, primary open-angle glaucoma.

* Adjusted for age, sex, ethnicity, hypertension, diabetes, body mass index, spherical equivalent, and intraocular pressure.
† Adjusted for age, sex, ethnicity, hypertension, diabetes, body mass index, spherical equivalent, and ocular perfusion pressure.
delivery of metabolic supply to RNFL, causing ganglion cell death and subsequently leading to glaucoma damage. In contrast, it was postulated that narrowing of retinal venules affects RNFL and development of glaucoma via more complex sequential events. Specifically, presence of narrowed retinal venule reflects venous congestion of the retinal microvasculature network, which may then lead to cytotoxic and vasogenic edema. These effects in turn lead to secondary constriction of retinal arterioles and further vascular dysregulation through venoarteriolar response, eventually leading to early RNFL thinning and subsequent glaucoma damage.

Validity of mediation analyses also depends on the core assumptions that there is no effect of the exposure that itself confounds the mediator-outcome relationship and that there is no interaction between the effects of the exposure and the mediator on the outcome. Based on our sensitivity analyses, the magnitude of association between RNFL thickness and glaucoma did not differ significantly with and without the adjustment of retinal vascular calibers (Supplementary Table S1). This suggests that the retinal vascular calibers (exposure) were unlikely to confound the association between the RNFL (mediating factor) and glaucoma (outcome). Furthermore, there was also no interaction effect between retinal vascular caliber and RNFL on glaucoma (data not shown). These observations indicated that the core assumptions for mediation analyses were unlikely to be violated in our analyses, further substantiating the validity of our findings.

On the other hand, there were also previous postulations which suggested that thinning of RNFL might be antecedent to retinal arteriolar narrowing in that thinner RNFL may have lower metabolic levels, and thus lower vascular demand, resulting in thinner vascular caliber. As an exploratory attempt to evaluate this possible reverse association in the interrelationship between retinal arteriolar caliber and RNFL thickness, we additionally performed a mediation analysis with RNFL thickness treated as the exposure and retinal arteriolar caliber as the mediator while adjusting for the same set of covariates as previous models (Supplementary Table S1). In the supplementary analysis, we observed that the mediation effect of RNFL thickness on glaucoma through retinal arteriolar caliber was not significant with a mere mediated proportion of approximately 3%. This is in contrast to the substantial mediated proportion of approximately 37% in the original mediation model which evaluated the effects of CRAE on glaucoma via RNFL thinning. Altogether, this statistical finding may weaken the plausibility of a reverse association between retinal vascular caliber and RNFL. Nevertheless, future experiments and longitudinal evaluations are still needed to further elucidate the causality relationships between retinal vessels and RNFL.

The strengths of our study include large population datasets across three Asian ethnicities, thus providing greater statistical power. Furthermore, our study included comprehensive measurements of potential confounding factors such as BP, diabetes, body mass index, spherical equivalent, and intraocular pressure. CI, confidence interval; CRAE, central retinal artery equivalent; CRVE, central retinal vein equivalent; OR, odds ratio; PM, proportion mediated; POAG, primary open angle glaucoma.

TABLE 4. Direct and Indirect Effects of Retinal Vascular Calibers on Glaucoma and Primary Open Angle Glaucoma

<table>
<thead>
<tr>
<th>Model</th>
<th>Glaucoma, n = 253</th>
<th>POAG, n = 195</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>P Value</td>
</tr>
<tr>
<td>CRAE, per SD decrease</td>
<td>Direct effect†</td>
<td>1.09</td>
</tr>
<tr>
<td></td>
<td>Indirect effect‡</td>
<td>1.02</td>
</tr>
<tr>
<td>CRVE, per SD decrease</td>
<td>Direct effect†</td>
<td>1.46</td>
</tr>
<tr>
<td></td>
<td>Indirect effect‡</td>
<td>1.03</td>
</tr>
</tbody>
</table>

All models were adjusted for age, sex, ethnicity, hypertension, diabetes, body mass index, spherical equivalent, and intraocular pressure. CI, confidence interval; CRAE, central retinal artery equivalent; CRVE, central retinal vein equivalent; OR, odds ratio; PM, proportion mediated; POAG, primary open angle glaucoma.

† Direct effect of CRAE/CRVE on glaucoma and additionally adjusted for retinal nerve fiber layer thickness.
‡ Indirect effect of CRAE/CRVE on glaucoma through retinal nerve fiber layer thickness.

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References


