Racial Differences in Retinal Vessel Geometric Characteristics: A Multiethnic Study in Healthy Asians

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Purpose. To investigate potential racial/ethnic differences in retinal vascular geometric parameters in a multiethnic Asian population (Chinese, Malay, and Indian) free of clinical diseases.

Methods. A series of retinal vascular parameters were measured from retinal photographs using a computer-assisted program following a standardized protocol. Healthy participants were defined as nonsmokers, the absence of diabetes mellitus, uncontrolled hypertension, obesity, stroke, heart disease, glaucoma, and retinopathy.

Results. There were significant differences in measurements of retinal vascular caliber, tortuosity, and fractal dimension among the three ethnic groups. In multiple linear regression model controlling for age, sex, body mass index, systolic blood pressure, cholesterol, and glucose levels, Indians had the largest arteriolar and venular calibers (arterioles [SE]: 158.94 μm [1.00]; venules: 228.26 μm [1.53]), followed by Malays (arterioles: 138.31 μm [0.74]; venules: 204.26 μm [1.13]), and then Chinese (arterioles: 131.20 μm [0.84]; venules: 195.09 μm [1.28]). Chinese had the largest arteriolar and venular tortuosity (arterioles [$\times 10^5$]: 7.20 [0.08]; venules [$\times 10^5$]: 9.09 [0.10]), and venular fractal dimension (1.244 [0.003]). There were no statistically significant differences in other retinal vascular parameters after correcting multiple comparisons by the method of modified false discovery rate.

Conclusions. We found that among ethnic groups composed of healthy Chinese, Malay, and Indians, there were statistically significant differences in several retinal parameters. There exist racial influences in retinal vascular parameters and other yet unknown or unmeasured environmental factor or lifestyle habits and genetic variations not related to race that may also contribute to these differences.

Keywords: retinal vasculature, epidemiology, false discovery rate

The retinal vasculature provides information on the health and disease status of the human circulation. There is increasing evidence that changes in retinal vasculature (e.g., retinal arteriolar narrowing and retinal venular widening) are early subclinical markers for major cardiovascular diseases (CVDs) (e.g., stroke) and their risk factors (e.g., hypertension and diabetes).[1–11] Such retinal vascular changes have also been shown to be linked with major eye diseases, including diabetic retinopathy,[5,12,13] glaucoma,[14] age-related macular degeneration,[15] and retinal vein occlusion.[16]

The characteristics and patterns of retinal vessels have been extensively described in several large epidemiologic studies.[17–22] Importantly, some studies have suggested possible racial/ethnic differences in retinal vascular characteristics.[7,22–24] For example, the Multi-Ethnic Study of Atherosclerosis (MESA) showed that compared with whites, blacks and Hispanics had larger retinal arteriolar calibers and blacks, Hispanics, and Chinese had larger retinal venular calibers.[7] Other studies suggest that iris pigmentation is a proxy for retinal pigmentation, which influences the measurements of retinal caliber that partly explained the observed ethnic differences in retinal vascular calibers.[20] Few studies, however, have analyzed in depth regarding the influence of race/ethnicity on retinal vascular measures, particularly newer retinal vascular parameters such as fractal dimension, branching characteristics (e.g., branching coefficient and angle) and length-to-diameter ratio (LDR).[25]

There are differences in the prevalence of CVD and its risk factors among different ethnicities. The Study of Health Assessment and Risk in Ethnic groups (SHARE) suggested that variation in conventional and novel risk factors between ethnic groups partly explains the higher rates of CVD among South

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Asians compared with Europeans and Chinese in Canada.[26] Therefore, understanding the effect of ethnicity on the variation of retinal vascular parameters may provide further significance in the prediction of CVD and eye diseases using retinal imaging technology.

The purpose of this study was to determine racial/ethnic differences on a range of retinal vascular measurements in a multiethnic healthy Asian adult population. In particular, we sought to minimize residual confounding by strict selection criteria and further model adjustment of confounders to determine the true impact of racial/ethnic differences on the retinal vasculature.

Materials and Methods

Study Population

A subgroup of healthy persons were randomly selected from three population-based studies: the Singapore Malay Eye Study (SiMES, 2004–2006), the Singapore Indian Eye Study (SINDI, 2007–2009), and the Singapore Chinese Eye Study (SCES, 2009–2011). An age-stratified random sampling frame was used to select ethnic Malays (78.7% response rate, $n = 3280$), Indians (75.6% response rate, $n = 3400$), and Chinese (72.8% response rate, $n = 3353$), 40 to 80 years of age, living in Singapore during each stipulated study period. The methodologies were published elsewhere in detail.[27,28] In brief, all participants underwent a comprehensive ocular examination. A detailed interviewer-administered questionnaire was used to collect relevant sociodemographic, lifestyle data, and medical history from all participants.[27] All of these studies were conducted at the Singapore Eye Research Institute, in accordance with the Declaration of Helsinki, with written informed consent obtained from all subjects before participation. The study protocols were approved by the SingHealth Institutional Review Board.

Fundus Examination

Digital fundus photography was taken using a 45° digital retinal camera (Canon CR-DGi with a 10D SLR digital camera backing; Canon, Tokyo, Japan) after pupil dilation using tropicamide 1% and phenylephrine hydrochloride 2.5%. Two retinal images of each eye were obtained, one centered at the optic disc and another centered at the fovea. The spatial resolution of each image was 3072×2048 pixels, and the images were stored without compression before analysis.

Measurement of Retinal Vascular Parameters

We used the optic disc–centered photograph of the right eye of each participant for image analysis; if the photograph of the right eye was ungradable, the measurement was performed on the left eye. Retinal vascular parameters were quantitatively measured and obtained from digital fundus images by using a new semiautomatic computer-assisted program (Singapore I Vessel Assessment [SIVA], version 3.0; National University of Singapore, Singapore, Singapore). Trained graders, masked to participant characteristics, executed the SIVA program to measure the retinal vasculature, according to a standardized protocol. The measured area of retinal vascular parameters was standardized and defined as the region from 0.5 to 2.0 disc diameters away from the disc margin. Images of poor quality, including those due to media opacities (e.g., dense lens opacity), small size of the pupil, or images that were out of focus or that had poor contrast, were excluded.

We measured the retinal vascular calibers following the standardized protocol used in the Atherosclerosis Risk in Communities Study.[29] On the basis of the revised Knudtson-Parr-Hubbard formula,[30] the retinal arteriolar and venular calibers were summarized as central retinal artery equivalent (CRAE) and central retinal vein equivalent (CRVE), respectively. We also measured the fractal dimension as a global summary measure of retinal vascular network pattern and geometry.[31–34] Larger values indicate a more complex branching pattern. Retinal vascular tortuosity was defined as the integral of the curvature square along the path of the vessel, normalized by the total path length.[35–38] A smaller tortuosity value indicates a straighter vessel.

A few parameters were measured to describe the properties of the branching of vessels, including branching coefficient (BC), branching angle (BA), and length-to-diameter ratio (LDR).[39–43] BC was calculated to measure the changes in the total cross-sectional area across the bifurcation. An increased BC represented wider branch vessels, and a decreased branching coefficient indicated narrower branch compared with the trunk vessel. BA was defined as the first angle subtended between the two daughter vessels at the vascular bifurcation.[44] LDR was defined as the ratio of the length between two branching points to the trunk vessel width.[45] The results of reliability tests have been reported previously.[42,46]

Definition of Healthy Individuals

We excluded the participants with any self-reported stroke, self-reported heart disease, diabetes mellitus, uncontrolled hypertension, obesity, current smoking, refractive error ≥ 8 diopters (D) or ≤ -12 D, glaucoma,[47] any retinal diseases (diabetic retinopathy[48] and age-related macular degeneration[49]), or had ungradable retinal fundus photographs. The definitions of glaucoma and retinal diseases have been reported previously.[47–49]

Uncontrolled hypertension was defined as self-reported physician diagnosed hypertension and use of antihypertension medication, whereas systolic blood pressure (SBP) was $\geq$140 mm Hg or diastolic blood pressure (DBP) was $\geq$90 mm Hg. Diabetes mellitus was defined as nonfasting plasma glucose $\geq$ 11.1 mmol/L, self-reported physician diagnosed diabetes, or use of glucose-lowering medication. Obesity was defined as body mass index (BMI) $\geq$ 30. Cigarette smoking was categorized into current smoking and noncurrent smoking (i.e., former or nonsmokers).

After exclusion, there were 231 Chinese, 277 Malays, and 151 Indians with available retinal vascular parameter data for analysis.

Socioeconomic Status

Socioeconomic factors such as educational level (primary or lower/secondary/postsecondary), individual monthly income (SGD$1000, SGD$1000–$2000, SGD$2000–$3000, and more than SGD$3000), and housing type (1- to 2-room flat/3- to 4-room flat/5-room flat, condominium, or private housing) were captured in our study.

Statistical Analyses

All statistical analyses were performed using R version 2.15.2 (available in the public domain at http://www.r-project.org/).[50] Characteristics of the study participants were expressed as mean (SD) or number (percentage). ANOVA or χ^2 test (or Fisher's exact test) was performed to test the parameter differences among Chinese, Malays, and Indians, as appropriate. We define the base model with covariates consisting of age, sex, BMI, SBP, total cholesterol, and glucose level as confounding variables. ANCOVA, which incorporates ANOVA and regression, was then performed to evaluate whether continuous retinal vascular parameters were equal across three ethnic groups, while further adjusting for the base model and three socioeconomic factors including education level, individual income, and housing. Such multiple covariates that adjusted for racial effects were displayed as adjusted mean (SE).

To further investigate and differentiate modifiable environmental effects (i.e., socioeconomic factors: education level, personal income, and housing) from racial effect on vascular parameters, we calculated partial eta squared,[51] which represents the proportion of the additional variability explained by each scioeconomic factor, its combined effect, and race, respectively, to the total variability unexplained by the base model described above. Partial eta squared can be used to compare the independent additional effect from each variable, where high partial eta squared indicates the higher explanatory impact of factor to the response variable. Likelihood ratio tests were applied to test the additional effects of each socioeconomic factor, socioeconomic factors combined, and race, respectively, from our base model.

A modified false discovery rate (FDR) technique in multiple testing under dependence was used to select important variables under the multiple testing situation of this analysis.[52] The adjustment based on FDR is able to identify markers more effectively than the conservative Bonferroni correction, which controls only the family-wise type I error.[53] FDR procedures are increasingly being used in research areas with large numbers of interrelated variables such as single nucleotide polymorphisms in population genetics[54] and voxels in neuroimaging.[55] Moreover, modified FDR is able to deal with dependent multiple tests, whereas the use of Bonferroni correction or naïve FDR approach requires the independence of the tests. The experiment-wise significance level (α) was set as 0.05.

Results

Table 1 summarizes the demographic characteristics of the study subjects. Overall, Indians were significantly older than Chinese or Malays ($P < 0.001$). There were significant differences in the distribution of age, sex, diastolic blood pressure, BMI, glucose, and low socioeconomic factors among the three ethnic groups. After correcting for multiple tests using the modified FDR method, age, sex, BMI, glucose, and low socioeconomic factors were still significantly different. Table 2 shows the comparison of retinal vascular parameters across the three ethnic groups. CRAE, CRVE, venular fractal dimension, and arteriolar and venular tortuosity were significantly different among the groups, after modified FDR correction. After further adjustment of multiple confounders, these five retinal parameters and arteriolar fractal dimension were statistically significantly different among the groups. Bifurcation parameters did not show significant differences among groups after correcting for multiple tests. Table 3 shows the relative proportion of variability explained by each social economic factor, its combination, and race that were unexplained by potential risk factors of age and sex, of BMI, SBP, total cholesterol, and glucose levels in the disease-free subjects. Race accounted for 32% of the unexplained variability for CRVE and over 40% for CRAE, arteriolar and venular tortuosity, whereas combined socioeconomic factors constitute less than 2.5%.

Figure 1 shows the racial effect on parameters selected from the statistically significant parameters after correcting for multiple comparisons in Table 2. Indians had the largest multiple adjusted mean [SE] of CRAE (158.94 μm [1.00]) and CRVE (228.26 μm [1.53]), followed by Malays (CRAE: 138.31 μm [0.74]; CRVE: 204.26 μm [1.13]), and then Chinese (CRAE: 131.20 μm [0.84]; CRVE: 195.09 μm [1.28]). Chinese had the

Table 1. Demographic Characteristics of the Study Subjects

Characteristics	Chinese, $n = 231$	Malay, $n = 277$	Indian, $n = 151$	*P* Value*
Age	52.75 (6.55)	50.53 (8.91)	55.93 (7.97)	<0.001†
Sex, female	113 (48.9%)	152 (54.9%)	48 (31.8%)	<0.001†
Systolic blood pressure	121.5 (10.79)	123.05 (9.77)	122.88 (10.94)	0.213
Diastolic blood pressure	74.56 (7.39)	73.29 (7.1)	75.08 (7.21)	0.028
BMI	22.78 (2.94)	24.5 (3.18)	24.75 (3.05)	<0.001†
Glucose	5.72 (1.66)	5.2 (1.14)	5.64 (1.24)	<0.001†
Cholesterol	5.55 (0.93)	5.45 (0.97)	5.33 (0.97)	0.097
Socioeconomic factors				
Education				<0.001†
Primary or lower	88 (38.1%)	140 (50.5%)	60 (39.7%)	
Secondary	63 (27.3%)	92 (33.2%)	53 (35.1%)	
Postsecondary	80 (34.6%)	45 (16.2%)	38 (25.2%)	
Income				<0.001†
<S$1000	57 (26%)	129 (46.7%)	46 (31.5%)	
S$1001–S$2000	62 (28.3%)	82 (29.7%)	50 (34.2%)	
S$2001–S$3000	41 (18.7%)	38 (13.8%)	19 (13%)	
≥S$3000	59 (26.9%)	27 (9.8%)	31 (21.2%)	
House				<0.001†
1- to 2-room flat	1 (0.4%)	26 (9.4%)	4 (2.7%)	
3- to 4-room flat	121 (52.6%)	178 (64.3%)	87 (58.8%)	
5-room flat/condominium/private housing	108 (47%)	73 (26.4%)	57 (38.5%)	

Data represented as mean (SD) or number (percentage).
* *P* value based on ANOVA or χ^2 test as appropriate.
† Significant after correcting multiple tests using modified FDR.

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Table 2. Summary of Retinal Vascular Parameters by the Three Ethnic Groups

Retinal Vascular Parameters	Chinese, *n* = 231	Malay, *n* = 277	Indian, *n* = 151	*P* Value*	*P* Value†
Caliber					
Central retinal artery equivalent	131.22 (9.99)	138.97 (10.07)	158.21 (18.17)	<0.001‡	<0.001‡
Central retinal vein equivalent	193.94 (13.85)	205.28 (15.82)	228.41 (25.23)	<0.001‡	<0.001‡
Fractal dimension					
Arterioles	1.25 (0.04)	1.22 (0.05)	1.24 (0.06)	0.041	<0.001‡
Venules	1.24 (0.05)	1.22 (0.05)	1.22 (0.06)	<0.001‡	<0.001‡
Tortuosity (×10^5)					
Arterioles	7.24 (1.27)	4.93 (0.99)	5.41 (1.36)	<0.001‡	<0.001‡
Venules	9.03 (1.3)	6.38 (1.16)	6.49 (1.86)	<0.001‡	<0.001‡
Bifurcation					
Branching coefficient [a]	1.42 (0.18)	1.41 (0.2)	1.46 (0.22)	0.055	0.659
Branching coefficient [v]	1.25 (0.16)	1.24 (0.14)	1.25 (0.19)	0.298	0.346
Branching angle [a]	79.46 (8.74)	79.64 (10.26)	80.45 (11.24)	0.620	0.059
Branching angle [v]	79.99 (8.94)	78 (9.29)	79.16 (9.8)	0.054	0.120
Length diameter ratio [a]	15.71 (7.56)	14.66 (7.97)	15.51 (7.69)	0.277	0.195
Length diameter ratio [v]	13.63 (7.42)	13.38 (7.93)	13.7 (7.18)	0.891	0.765

Data represented as mean (SD); [a], arterioles; [v], venules.
* *P* value based on ANOVA.
† *P* value based on ANCOVA, adjusting for age, sex, body mass index, systolic blood pressure, cholesterol and glucose, educational level, individual income, and housing type.
‡ Significant after correcting multiple tests using modified FDR.

largest tortuosity (arterioles (×10^5): 7.24 [0.09]; venules (×10^5): 9.09 [×0.10]), and venular fractal dimension (1.244 [0.003]). Malays and Indians had similar tortuosity and venular fractal dimension, whereas Chinese and Indians had similar arteriolar fractal dimension.

Figure 2 shows examples of retinal fundus photographs assessed quantitatively by the Singapore I Vessel Assessment (SIVA) software (version 3.0; National University of Singapore) for fractal dimension of age- and sex-matched Chinese, Malay, and Indian participants.

Table 3. Residual Analysis to Study the Contribution of Each Factor on Response

Parameter	Socioeconomic Status				Race
	Education	Income	House	Combined†	
Caliber					
Central retinal artery equivalent					
Partial eta squared, %	1.1	0.55	0.51	1.55	42.9
P value*	0.03	0.323	0.201	0.193	<0.001
Central retinal vein equivalent					
Partial eta squared, %	1.18	0.71	0.15	1.68	31.8
P value*	0.023	0.21	0.627	0.15	<0.001
Fractal dimension					
Arterioles					
Partial eta squared, %	0.01	0.62	0.22	1.14	5.4
P value*	0.968	0.268	0.506	0.4	<0.001
Venules					
Partial eta squared, %	0.21	0.22	0.17	0.82	3.77
P value*	0.512	0.711	0.576	0.631	<0.001
Tortuosity					
Arterioles					
Partial eta squared, %	0.41	1.44	1.66	2.44	40.09
P value*	0.273	0.027	0.005	0.029	<0.001
Venules					
Partial eta squared, %	0.49	1.63	0.16	1.69	40.87
P value*	0.212	0.016	0.607	0.149	<0.001

* Likelihood ratio test.
† Education, Income, and Housing combined.

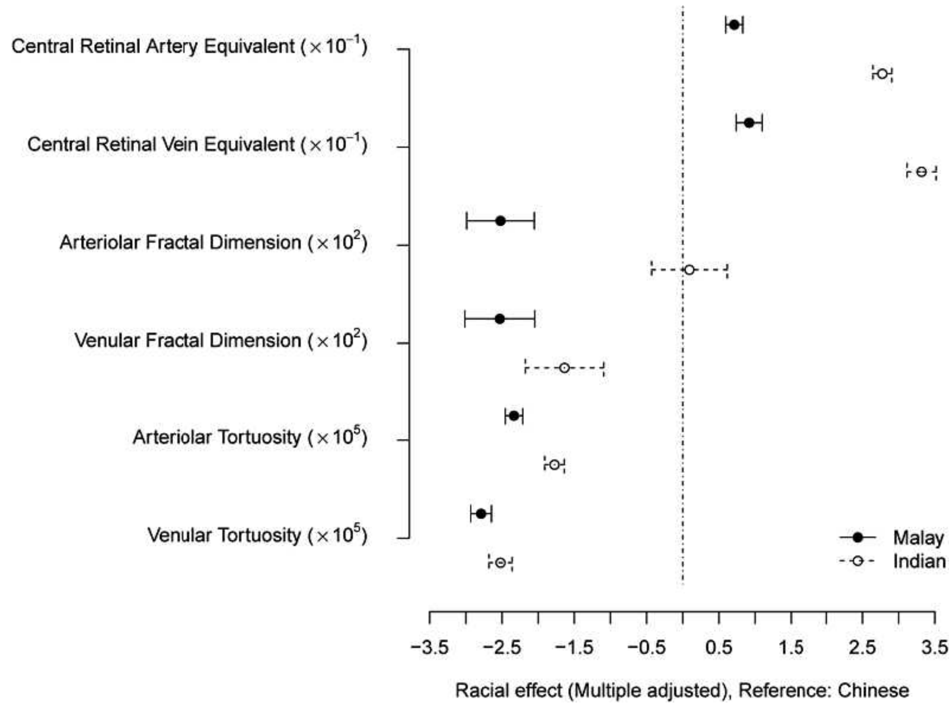

Figure 1. Racial difference of retinal vascular parameters, adjusting for age, sex, body mass index, systolic blood pressure, cholesterol, and glucose. The data presented are mean and SEs. Chinese serve as "reference," and the racial effect is represented as the difference between Chinese and two other ethnic groups. Some numbers were multiplied by 10, to be shown in one graph regardless of the parameter units.

Discussion

Few studies have directly compared a wide range of retinal vascular parameters across multiethnic populations, particularly in healthy adult populations. With a wide spectrum of retinal vascular parameters, our study allowed us to perform a comprehensive comparison among three ethnic groups and provided new insight into potential differences in retinal vascular parameters among different ethnic groups. We observed that retinal vascular parameters, in particular retinal vascular calibers, fractal dimension, and tortuosity, were statistically significantly different among Chinese, Malays, and Indians after correcting for multiple tests using the modified FDR approach.

Our main limitation in the interpretation of our findings is the methodologic issue of addressing residual confounding, which may underlie some of our observed differences. Despite our efforts to limit confounding by implementation of strict exclusion criteria and adjustment in the analyses, the differences in retinal vascular parameters among different ethnicities may still be due to residual confounding. However, our residual analysis showed high racial impact on vascular parameters (accounted for 32% of the unexplained variability for CRVE and over 40% for CRAE, and arteriolar and venular tortuosity), of up to almost 30-fold over the impact of modifiable combined socioeconomic factors (i.e., education level, personal income, and housing). Nonetheless, there are other underlying mechanisms such as genetic variations and lifestyle habits not related to race or other environment factors yet unknown or unmeasured (i.e., diet or preventive medical care) in our present study that may influence the reported differences. Ethnic variability in the retinal vessel calibers,

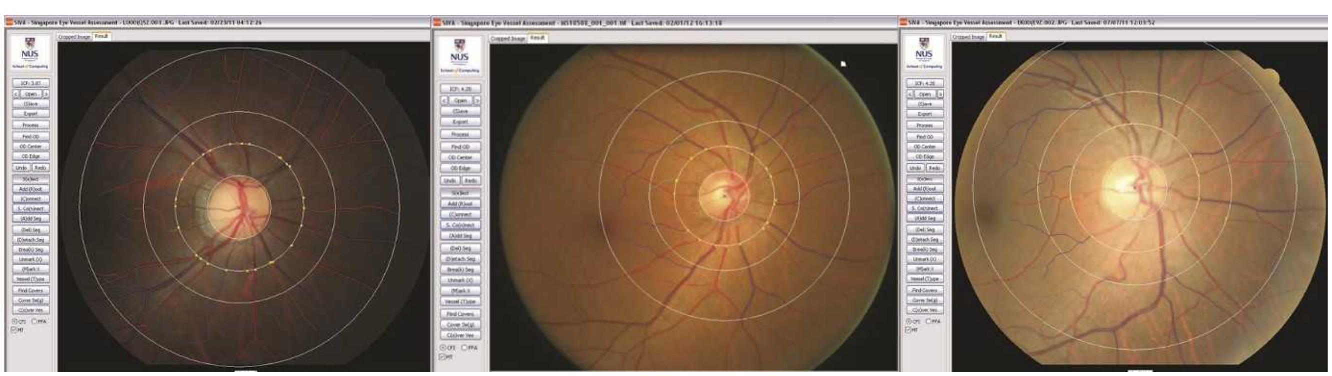

Figure 2. Retinal fundus photograph assessed quantitatively by a newer computer-assisted program. Arterioles are in *red* and venules are in *blue*. The measured area of retinal vascular fractal dimension was standardized and defined as the region from 0.5 to 2.0 disc diameters away from the disc margin. Fractal dimension was calculated from the *skeletonized line tracing* using the box-counting method. Three examples are presented. *Left*: a Chinese person (45 years of age; female) with fractal dimension (arteriole: 1.2808; venules: 1.2775). *Middle*: a Malay person (44 years of age; female) with fractal dimension (arterioles: 1.2088; venules: 1.1929). *Right*: an Indian person (43 years of age; female) with fractal dimension (arterioles: 1.2771; venules: 1.2272).

which may also be partly due to the variations in retinal pigmentation, as approximated by iris color, could be a possible source of measurement error of retinal vessel caliber.[20] Other possible limitations include selection bias due to unmatched data despite our strict selection criteria, bias from self-reported measures, and random errors associated with photographic technique and cardiac cycle.[24,29] The strengths of this study include the use of a computer-assisted program and the quantitative measurement on a range of retinal vascular parameters.

Numerous studies have reported a strong link between changes in retinal vascular caliber and a range of systemic factors (e.g., hypertension and diabetes), suggesting that retinal vascular caliber measurement can be used as a research tool to better understand the relationship between the retinal microvasculature and systemic diseases.[1–3,7,56] Recent studies have identified a number of other retinal vascular features including tortuosity, fractal dimension, bifurcation, and LDR that may also relate to microvascular damage and indicate the "optimal state" of the retinal microcirculation. For example, retinal vascular tortuosity was shown to be positively associated with cholesterol and blood pressure levels.[5,22] Other studies suggested that retinal vascular fractal dimension is association with hypertension[42,57] and stroke.[58,59] Thus, retinal vascular imaging technology may help to shed important clinical implications for the prediction, prevention, or treatment of CVD and major eye diseases. Testing and validating the clinical use of retinal vascular imaging for CVD risk prediction and risk stratification are currently ongoing.[25]

There is growing evidence to imply that retinal vascular parameters may vary between racial and ethnic groups. The MESA has shown that compared with whites, blacks and Hispanics had larger CRAE; and blacks, Hispanics, and Chinese had larger CRVE.[7] The Sydney Childhood Eye Study (SCES) reported that both CRAE and CRVE were substantially wider in East Asian than those in the Caucasian children.[20] The Singapore Cohort Study of the Risk Factors for Myopia (SCORM) reported CRVE and CRAE were significantly narrower in Chinese, compared with Malay and Indian children.[24] The Child Heart and Health Study in England (CHASE) found that South Asian (Indian, Pakistani, Bangladeshi, or a combination of these) and African Caribbean children had levels of arteriolar tortuosity similar to those of white Europeans, whereas other ethnic groups from Asia (mainly Afghanistan, China, and Turkey) had lower levels of arteriolar tortuosity.[22] In accordance with these findings, we found that Chinese had narrower caliber and higher tortuosity compared with those of Malays and Indians.

Our data suggested that fractal dimension varied between ethnic groups. Chinese had larger venular fractal dimension compared with that of Malays and Indians, and Malays had smaller arteriolar fractal dimension compared with that of Chinese and Indians. However, no significant difference was found in branching parameters after controlling for multiple comparisons using modified FDR.

In conclusion, our findings suggest racial differences in retinal vascular parameters, in particular, retinal vascular calibers, fractal dimension, and tortuosity in a healthy multiethnic Asian adult population. Other yet unknown factors or unmeasured environmental factor or lifestyle habits or genetic variations not related to race may also explain some of these differences.

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