Familial Correlation of Retinal Vascular Caliber in Singapore Chinese

Ling-Jun Li,[1] Jiemin Liao,[2] Qiao Fan,[3] Carol Yim-lui Cheung,[1,2,4] M. Kamran Ikram,[1,2,5,6] Ching-Yu Cheng,[1–4] Seang-Mei Saw,[1,3] and Tien-Yin Wong[1–4]

[1]Singapore Eye Research Institute, Singapore National Eye Centre, Singapore
[2]Department of Ophthalmology, National University Health System, National University of Singapore, Singapore
[3]Saw Swee Hock School of Public Health, National University of Singapore, Singapore
[4]Centre for Quantitative Medicine, DUKE-NUS Graduate Medical School, Singapore
[5]Memory Aging and Cognition Centre, National University of Singapore, Singapore
[6]Department of Ophthalmology, Erasmus Medical Centre, Rotterdam, The Netherlands

Correspondence: Tien-Yin Wong, Singapore Eye Research Institute, Singapore National Eye Centre, 11 Third Hospital Avenue, Singapore 119228; wong.tien.yin@snec.com.sg.

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Purpose. Our study aimed to explore the heritability of retinal vascular caliber among Singapore Chinese families.

Methods. In the Strabismus, Amblyopia, and Refractive Error Study in Singaporean Chinese Preschoolers (STARS) family study conducted from 2008 to 2010, a total of 727 participants (304 parent-child pairs, 83 sibling pairs, and 87 spouse pairs) were included in the analysis. According to standardized protocols, retinal photography, blood pressure measurements, anthropometric measurements, and interviews were performed at clinic. Retinal vascular caliber was assessed by a computer-assisted imaging program (IVAN). Familial correlation of retinal vascular caliber among family pairs was calculated by the FCOR procedure with S.A.G.E. computer software program package and heritability was double the value of the familial correlation.

Results. Mean age was 8.59 years in 304 children and 39.90 years in 423 parents. Mean CRAE and CRVE were 157.09 and 220.80 μm in children, and 150.29 and 220.70 μm in parents, respectively. In multivariate analysis, familial correlation of CRVE was 0.36 ($P < 0.001$) among parent-child pairs and 0.28 ($P < 0.05$) among sibling pairs, respectively. Heritability of CRVE was 0.72 and 0.56 among parent-child pairs and sibling pairs, respectively. Family correlation and heritability of CRAE were not significant.

Conclusions. This familial correlation study showed a strong correlation of retinal venular caliber in Singapore Chinese families among parent-child pairs and sibling pairs, independent of age, sex, blood pressure, and BMI. Our findings provide further evidence on substantial heritability of the microvasculature.

Keywords: familial correlation, retinal vascular caliber, Singapore Chinese

In the past decade, major genetic studies have identified genetic markers for a range of systematic vascular risk factors (e.g., hypertension, obesity),[1–4] cardiovascular diseases (CVD),[5–9] and type 2 diabetes.[2] In contrast, there are fewer studies on the heritability and genetics of the microcirculation, increasingly thought to be a major pathway for CVD.

The retinal blood vessels can be assessed noninvasively and quantitatively with advances in retinal photography and imaging software.[10–13] Epidemiological and clinical studies have shown that changes in the caliber of the retinal vessels (e.g., retinal arteriolar narrowing, retinal venular widening) are related to hypertension, diabetes, stroke, and CVD.[14–18] Thus, the assessment of genetic determinants of the retinal vasculature may offer unique opportunities to identify new genes for these systemic risk factors and diseases. In a genome-wide association study (GWAS) of Caucasian subjects, new loci for retina venular caliber were identified that provided insights into the genetic determinants for CVD.[19]

It is increasingly recognized that genetic markers of diseases are different between Caucasian and Asian persons.[20] Currently there is no study on the genetics of the retinal vasculature in Asian populations, and indeed, it is unclear if genes contribute significantly to the morphology of the retinal vasculature in Asian individuals. Because heritability is generally regarded as the initial step to identify genetic traits,[21] our study aimed to investigate the familial correlation of retinal vascular caliber among Singapore Chinese children and their family members.

Methods

Study Design

The Strabismus, Amblyopia and Refractive Error Study in Singaporean Chinese Preschoolers (STARS) Family study is a family-based study nested in a prevalence survey conducted from March 2008 to March 2010.[22] It is a family-based genetic study of early-onset myopia probands in STARS. The biological parents and siblings of STARS probands with myopia were invited to the STARS Family study. A total of 895 subjects were screened, whereas only 592 children aged 4 to 16 years were

recruited (response rate 66.1%). Retinal photography and clinical examination were performed on 304 children (51.4%) and 423 related parents, which resulted in 304 parent-child pairs, 83 full sibling pairs, and 87 spouse pairs, with a total number of 727 participants.

The STARS Family study followed the tenets of the Declaration of Helsinki and was approved by Institutional Review Board of the Singapore Eye Research Institute (SERI) and the National Healthcare Group (NHG). Informed written consent was obtained from parents after explanation at clinic.

Retinal Photography and Measurement of Retinal Vascular Caliber

The subjects were examined either at SERI or Jurong Medical Center. Pupil dilation was conducted on both eyes among parents and children, followed by the 45° digital retinal photography examination by using standardized settings of a Canon camera (Model CR6-NM45; Canon, Inc., Tokyo, Japan). Digitized retinal images centered on the optic disc were taken and further sent for assessments according to a standard protocol described in our earlier articles.[22–24] The semi-automated computer imaging program, IVAN (University of Wisconsin, Madison, WI), was used to measure the retinal vessel width within the area of 0.5 to 1.0 disc diameters away from the disc margin (Zone B). Based on the revised Knudtson-Parr-Hubbard formula,[25] retinal arteriolar and venular caliber was summarized as central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE), respectively. The ratio of CRAE over CRVE is defined as arteriolar-to-venule ratio (AVR). Because both eyes are highly correlated, only one eye's readings were used. According to standard protocol, we intended to use data from the right eye retinal photograph as first choice, and to use the left eye photograph only if the right eye photograph was not gradable. However, in our study, all participants' right eye retinal photographs were gradable, so the data presented are all based on right eyes.

Due to the semiautomation of IVAN, the grader needs to not only use the program but also evaluate and define adjustment to the measurements (e.g., deselect wrong vessels and select appropriate ones), which has been well established.[22–24] Thus, a single trained grader, masked to participant characteristics, measured retinal vascular caliber from all retinal fundus photographs in this study. "Ungradable photograph" is defined as retinal photographs that were poorly focused or had one quadrant cut off. Intragrader reliability was assessed in 60 (8%) randomly selected retinal photographs from the STARS Family study. The intraclass correlation coefficient was 0.90 for CRAE and 0.94 for CRVE.

Other Information

Blood pressure was measured using the automatic Omron sphygmomanometer (Omron HEM 705 LP; Omron Healthcare, Inc., Schaumburg, IL) with an appropriate pediatric cuff size, after 5 minutes of rest. Two separate measurements were taken and their average was calculated.[24] A third attempt was added if the difference between the first two readings were greater than 10 mm Hg in systolic blood pressure (SBP) and/or 5 mm Hg in diastolic blood pressure (DBP). The average of the two closest readings was used for analysis. Mean arterial blood pressure (MABP) was calculated as one-third of SBP plus two-thirds of DBP.

Height and weight were both measured while barefoot and in the upright standing position, according to a standard protocol of SECA weighing scale (Vogel and Halke, Hamburg, Germany).[23] Height was recorded to the nearest 1.0 mm while weight was recorded to the nearest 0.1 kg. Two readings of each were required. Body mass index (BMI) was calculated as the average reading of weight divided by the average reading of height squared (kilograms per meter squared).

Table 1. Baseline Characteristics of STARS Family Participants

	Children, $n = 304$		Parents, $n = 423$	
Variables	**Mean**	**SD**	**Mean**	**SD**
Age, y	8.59	3.86	39.90	5.42
Sex, male %	45.07	—	50.35	—
MABP, mm Hg	78.60	10.08	89.91	11.68
BMI, kg/m^2	17.12	3.20	24.31	4.28
CRAE, μm	157.09	14.51	150.29	17.14
CRVE, μm	220.80	20.91	220.70	21.57
AVR	0.71	0.07	0.68	0.07

Statistical Analysis

Familial correlation of retinal vascular caliber among family pairs was calculated by Family Correlation (FCOR) procedure with the S.A.G.E. (Statistical Analysis for Genetic Epidemiology; Statistical Solutions, Ltd., Cork, Ireland) computer software program package.[26–29] To obtain estimates of familial correlations adjusted for covariates, phenotypic residuals from regression models were used as input. Linear regressions were performed using STATA software, version 12.1 (STATA Corp., College Station, TX), with each phenotype as a dependent variable and covariates as independent variables. The phenotypic residuals were computed as the difference between the original and the predicted phenotype measurements after accounting for covariates. Mean of correlations and SEs were analyzed first after adjusting for age and sex. To reduce the residual effects and variation in phenotype of retinal vascular caliber due to blood pressure and BMI, both of which have been identified as risk factors for retinal vascular caliber,[23,24,30–33] we further adjusted for MABP and BMI in our familial correlation analysis. Heritability, estimated by doubling the adjusted correlation reported in single parent-child pairs and full sibling pairs, was suggested to be high if the value was above 0.3.[21] P value for correlation was indicated.

To take ocular magnification from the fundus camera into account, we corrected CRAE and CRVE for additional familial correlation analysis. The formula applied to this study was suggested by Bengtsson and Krakau[34] as corrected CRAE/CRVE = crude CRAE/CRVE * (1 − 0.0017*SE).

Results

A total of 304 children and 423 parents were included in the final analysis. The baseline characteristics of both groups are described in Table 1 and all variables were approximately normally distributed. Means (SDs) of age, CRAE, and CRVE were 8.59 (3.86) years, 157.09 (14.51) μm, and 220.80 (20.91) μm for children; 39.90 (5.42) years, 150.29 (17.14) μm, and 220.70 (21.57) μm for parents, respectively.

Correlation of CRAE and CRVE among 304 parent-child pairs, 83 sibling pairs, and 87 spouse pairs are shown in Table 2. In Table 2, the correlation of CRAE and CRVE was 0.12 ($P < 0.05$) and 0.31 ($P < 0.001$) among single parent-child pairs after adjusting for age and sex, whereas only the correlation of CRVE remained significant as 0.37 ($P < 0.001$) after further adjusting for MABP and BMI. Thus, the heritability of CRVE among parent-child pairs was as high as 0.74 in multivariate analyses. The correlation was found only in CRVE among sibling pairs in both models. After adjusting for age, sex, MABP,

Table 2. Familial Correlation of Retinal Vascular Caliber Between Parents and Children

	n	CRAE Correlation, SE	CRVE Correlation, SE	AVR Correlation, SE
Parent-child pair	304			
Model 1		0.12, 0.07*	0.31, 0.07†	0.08, 0.07
Model 2		0.09, 0.08	0.37, 0.07†	0.06, 0.08
Sibling pair	83			
Model 1		0.16, 0.12	0.26, 0.11*	0.08, 0.12
Model 2		0.21, 0.13	0.28, 0.13*	0.01, 0.13
Spouse pair	87			
Model 1		0.18, 0.11	0.19, 0.10	−0.004, 0.11
Model 2		0.19, 0.11	0.18, 0.11	0.05, 0.11

Model 1: adjusted for age and sex; Model 2: adjusted for age, sex, MABP, and BMI.
* $P < 0.05$.
† $P < 0.001$.

and BMI, correlation of CRVE was 0.28 ($P < 0.05$) in sibling pairs. According to the high variance of 0.11, there was no significant familial correlation among spouse pairs even though it was reported as high as 0.18 in our study. In the additional analysis in which we corrected CRAE and CRVE for ocular magnification, familial correlation was similar between the corrected and uncorrected CRAE/CRVE values among single parent-child pairs (0.38 [SE = 0.10] vs. 0.37, [SE = 0.07], both $P < 0.001$) and sibling pairs (0.29 [SE = 0.13] vs. 0.28 [SE = 0.13], both $P < 0.05$), respectively. Therefore, we concluded that myopia might not significantly affect the familial correlation observed in our family. To keep our finding comparable to the Beaver Dam Eye study (BDES), we opted for using the uncorrected CRAE and CRVE values.

To rule out any possible identifiable subgroup differences from others in our correlations, we further tested the homogeneity between subgroups in our parent-child pairs and sibling pairs (Table 3). The homogeneity test of CRVE was not significant either in parent-child pairs ($P = 0.50$) or sibling pairs ($P = 0.56$) after adjusting for age, sex, MABP, and BMI, which suggested that the correlation was similar among the subgroup of family pairs, where subgroups are defined as brother-sister combinations for sibling pairs and father, mother, son, and daughter combinations for parent-child pairs.

To investigate the shared genetic and environmental components for the related traits, we performed a further analysis on phenotypic correlations between traits of CRVE*-CRAE, CRVE*MABP, CRVE*BMI, CRAE*MABP, and CRAE*BMI (Table 4). High to moderate phenotypic correlations were found in CRVE*CRAE (0.52, $P < 0.001$), CRAE*MABP (−0.26, $P < 0.001$), and CRVE*BMI (0.10, $P = 0.02$). In other trait pairs, CRVE*MABP and CRAE*BMI, overall phenotypic correlations were too low to be significant.

After taking ocular magnification from the fundus camera into account, myopia was suggested to be associated with retinal caliber in some studies,[22,35] but not in others.[36,37] Accordingly, we tried to correct only for ocular magnification instead of adjusting for refractive error in our study. The formula as "corrected CRAE/CRVE = crude CRAE/CRVE * (1 − 0.0017*SE)"[34] was used in our study and familial correlation was reanalyzed. In the new results, familial correlation was similar between the corrected and uncorrected CRAE/CRVE values among single parent-child pairs (0.38 [SE = 0.10] vs. 0.37, [SE = 0.07], both $P < 0.001$) and sibling pairs (0.29 [SE = 0.13] vs. 0.28 [SE = 0.13], both $P < 0.05$), respectively.

Discussion

In this study among Chinese persons, we found high heritability of retinal venular caliber among parent-child pairs and sibling pairs that was not seen in spouse pairs, consistent with the concept of underlying genetic influence running in the family for venular caliber. To our best knowledge, our study is the first to investigate possible genetic influences on the microcirculation via familial correlation among Chinese persons.

In the BDES, which comprised white persons, correlation of retinal vascular caliber existed in relatives but not in unrelated individuals. Among relatives, direct blood line had higher correlation than indirect blood line (parent-child > siblings > avuncular > cousins > spouse). For example, correlations of parent-child pairs and cousin pairs were 0.24 and 0.08 in CRVE, and 0.27 and 0.06 in CRAE, respectively.[21] The spousal correlation in retinal vascular caliber was only 0.03 in CRVE and 0.05 in CRAE, as there was no genetic correlation between husbands and wives. In our study, a similar trend was established in the Singapore Chinese families. After adjusting

Table 3. Homogeneity Tests for Correlation of Retinal Vascular Caliber

	Between Subgroups			Between Traits
	CRAE *P* Value	CRVE *P* Value	AVR *P* Value	CRAE vs. CRVE *P* Value
Parent-child pair				
Model 1	0.24	0.25	0.95	0.62
Model 2	0.67	0.50	0.82	0.81
Sibling pair				
Model 1	0.77	0.47	0.42	0.68
Model 2	0.99	0.56	0.67	0.68

Model 1: adjusted for age and sex; Model 2: adjusted for age, sex, MABP, and BMI.

Table 4. Overall Phenotypic Correlation Between Related Traits of CRAE, CRVE, MABP, and BMI

Trait 1	Trait 2	Overall Phenotypic Correlation*	*P* Value
CRVE	CRAE	0.52	<0.001
CRVE	MABP	−0.04	0.40
CRVE	BMI	0.10	0.02
CRAE	MABP	−0.26	<0.001
CRAE	BMI	−0.07	0.10

* Adjusted for age and sex.

for age, sex, MABP, and BMI, the familial correlation of CRVE was 0.36 in parent-child pairs, 0.28 in sibling pairs, and 0.18 in spouse pairs, accordingly. Our finding is consistent with genetic influence showing similar parent-child and sibling correlations (who shared 50% of their genes), about half the parent-child correlations for avuncular correlations (25% of their genes), and about half again for cousin correlations (12.5% of their genes). Our findings are also consistent with other studies. In the Danish Twin Study, Taarnhoj et al.[38,39] found heritability was accordingly 70%, 83%, and 82% in retinal arteriolar caliber, retinal venular caliber, and retinal arteriolar tortuosity, whereas only 18% variance in retinal arteriolar tortuosity was explained by environmental risk factors. In the UK Twin Project[40] and the Australian Twin Eye Study,[41] researchers also found up to 60% of covariance in genetic variance of both retinal arteriolar and venular caliber, with approximately 30% of covariance in environmental risk factors and only 5% of combined associated CVD risk factors. Many studies have shown that retinal vascular changes are associated with a range of major vascular and metabolic diseases, such as hypertension,[42] type 2 diabetes,[43] CVD,[44] and stroke.[15,17,18,45] Our study thus suggests that further studying genetics of retinal vascular caliber may provide new insights into shared genetic control of microvascular pathways and systemic vascular diseases.

The overall phenotypic correlation between relative traits showed that CRAE and CRVE were highly correlated at 0.52, due to strong genetic and environmental correlation. The phenotypic correlation between CRAE and MABP was −0.26, and largely attributed to shared genetic factors. Our findings suggested that MABP had genetic sharing with CRAE, whereas both MABP and BMI had no genetic sharing with CRVE, consistent with the Guangzhou Twin Eye Study results.[46] This may explain why our familial correlation of CRAE among parent-child pairs was attenuated after further adjusting for MABP. Furthermore, two recent studies (GWAS and admixture mapping in chromosomal regions study, Ikram et al.[19] and Cheng et al.[47]) had reported possible loci in retinal arteriolar caliber (8p23.1) and retinal venular caliber (2q14, 6q21.1, 19q13, 6q24, 12q24, and 5q14), based on a large number of Caucasian and African populations. These genetic findings suggested a greater number of inherited loci on retinal venular caliber than retinal arteriolar caliber, consistent with higher heritability for CRVE.

The strength our study is that our participants tended to be younger and healthier, free from overt systemic diseases or ocular diseases, compared with previous studies. However, our study has some limitations. First, no gene-environment interaction was examined. Second, the missing data on blood pressure measurement, BMI measurement, and past of the parent participants might attenuate the true effect of familial correlation after multivariate analysis. Third, CRAE and CRVE were not corrected for refractive error and other potential confounders; thus, residual effects were not thoroughly accounted for.

In conclusion, our familial correlation study in Asian Chinese showed a strong correlation of retinal venular caliber in direct blood line relatives, such as parent-child pairs and sibling pairs, independent of age, sex, blood pressure, and BMI, providing the heritability evidence of genetic determination on retinal vessel morphology.

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