

Hypertension

JOURNAL OF THE AMERICAN HEART ASSOCIATION



*Learn and Live*SM

Quantitative Genetic Analysis of the Retinal Vascular Caliber: The Australian Twins Eye Study

Cong Sun, Gu Zhu, Tien Y. Wong, Alex W. Hewitt, Jonathan B. Ruddle, Lauren Hodgson, Grant W. Montgomery, Terri L. Young, Christopher J. Hammond, Jamie E. Craig, Nicholas G. Martin, Mingguang He and David A. Mackey

Hypertension 2009;54;788-795; originally published online Aug 17, 2009;

DOI: 10.1161/HYPERTENSIONAHA.109.132902

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75214

Copyright © 2009 American Heart Association. All rights reserved. Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://hyper.ahajournals.org/cgi/content/full/54/4/788>

Data Supplement (unedited) at:

<http://hyper.ahajournals.org/cgi/content/full/HYPERTENSIONAHA.109.132902/DC1>

Subscriptions: Information about subscribing to Hypertension is online at
<http://hyper.ahajournals.org/subscriptions/>

Permissions: Permissions & Rights Desk, Lippincott Williams & Wilkins, a division of Wolters Kluwer Health, 351 West Camden Street, Baltimore, MD 21202-2436. Phone: 410-528-4050. Fax: 410-528-8550. E-mail:
journalpermissions@lww.com

Reprints: Information about reprints can be found online at
<http://www.lww.com/reprints>

Quantitative Genetic Analysis of the Retinal Vascular Caliber

The Australian Twins Eye Study

Cong Sun, Gu Zhu, Tien Y. Wong, Alex W. Hewitt, Jonathan B. Ruddle, Lauren Hodgson, Grant W. Montgomery, Terri L. Young, Christopher J. Hammond, Jamie E. Craig, Nicholas G. Martin, Mingguang He, David A. Mackey

Abstract—Research into the genetic effects and specific genes associated with retinal vascular caliber, a risk marker of cardiovascular diseases, may provide new insights into the genetic contribution of early microvascular disease. A combined 374 monozygotic and 536 dizygotic twin pairs and 322 siblings from the Twins Eye Study in Tasmania and the Brisbane Adolescent Twin Study underwent complete ophthalmic examinations, including retinal photography, and bilateral retinal vascular caliber was measured. Structural equation modeling was used to estimate the heritability. Genome-wide linkage analysis was conducted on 836 individuals from 381 Brisbane Adolescent Twin Study families, with adjustments for age, sex, and other covariates. The heritabilities for the retinal arteriolar caliber were 59.4% (95% CI: 53.2% to 64.7%) and 56.5% (95% CI: 50.1% to 61.9%) in the Twins Eye Study in Tasmania and the Brisbane Adolescent Twin Study, respectively, and for venular caliber they were 61.7% (95% CI: 55.6% to 67.0%) and 64.2% (95% CI: 58.7% to 68.8%), respectively, after adjusting for age, sex, and body mass index. Two multipoint peaks detected on chromosomes 3p12.3 and 8p23.1 for retinal arteriolar caliber had suggestive linkage, with the highest multipoint peak logarithm of odds score of 2.24 on chromosome 8p23.1 (genome-wide $P=7.0\times 10^{-4}$). Two suggestive logarithm of odds scores for venular caliber were identified on chromosomes 2p14 and 9q21.13. The largest multipoint logarithm of odds score was 2.69 on chromosome 2p14 (genome-wide $P=2.0\times 10^{-4}$). In this large twin population, genetic factors appear to play a significant role in the variation of retinal vascular caliber. Several putative loci were identified for the retinal vascular caliber. (*Hypertension*. 2009;54:788-795.)

Key Words: twins ■ heritability ■ retinal vascular caliber ■ genome-wide linkage analysis ■ genetics

Recent population studies suggest that a quantitative assessment of retinal vascular caliber may allow understanding of early structural changes in the microcirculation and the relationship of these changes to the risk of hypertension, diabetes mellitus, and cardiovascular disease.¹⁻⁵ Variation in retinal arteriolar and venular calibers appears to reflect differential effects of systemic, environmental, and possibly genetic factors.¹ For example, narrower retinal arterioles are associated with higher levels of past, current, and future blood pressure and obesity and predict the incidences of diabetes mellitus and coronary heart disease.⁶⁻¹² In contrast, wider retinal venules are associated with impaired fasting glucose metabolism, dyslipidemia, obesity, inflammation, endothelial dysfunction, and cigarette smoking, and predict the risks of stroke and coronary heart disease.¹³⁻¹⁸

Data from a familial aggregation study,¹⁹ a twin study,²⁰ and a genome-wide linkage study²¹ suggest that genetic

factors contribute substantially to the normal variation in retinal vascular caliber. Several putative loci have also been identified.²¹ Research into the genetic effects and specific genes associated with retinal vascular caliber may provide new insights into the genetic contribution of early microvascular disease.^{1,22,23}

In the current study, we examined the heritability of retinal vascular caliber in a sample of Australian twins ranging in age from 5 to 90 years. We then performed a genome-wide linkage scan in a subsample of 836 individuals from 381 families to identify underlying quantitative trait loci (QTL).

Methods

Study Population

The study populations were derived from predominately white twins, recruited from the Twins Eye Study in Tasmania (TEST), which included twins from the Tasmanian Infant Health Cohort and the

Received March 19, 2009; first decision April 19, 2009; revision accepted July 21, 2009.

From the Centre for Eye Research Australia (C.S., T.Y.W., A.W.H., J.B.R., L.H., D.A.M.), University of Melbourne, Melbourne, Victoria, Australia; Queensland Institute of Medical Research (G.Z., G.W.M., N.G.M.), Brisbane, Queensland, Australia; Singapore Eye Research Institute (T.Y.W.), Yong Loo Lin School of Medicine, National University of Singapore, Singapore; Duke Eye Center (T.L.Y.), Duke University Medical Center, Duke University, Durham, NC; Department of Twin Research and Genetic Epidemiology Unit (C.J.H.), King's College London, London, United Kingdom; National Health and Medical Research Council Centre for Clinical Eye Research (J.E.C.), Department of Ophthalmology, Flinders University, Flinders Medical Centre, Adelaide, South Australia, Australia; State Key Laboratory of Ophthalmology (M.H.), Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, China; Discipline of Medicine (D.A.M.), University of Tasmania, Tasmania, Australia.

Correspondence to David A. Mackey, Centre for Eye Research Australia, University of Melbourne, Department of Ophthalmology, Royal Victorian Eye and Ear Hospital, 1/32 Gisborne St, East Melbourne, Victoria 3002, Australia. E-mail D.Mackey@utas.edu.au

© 2009 American Heart Association, Inc.

Hypertension is available at <http://hyper.ahajournals.org>

DOI: 10.1161/HYPERTENSIONAHA.109.132902

Australia Twin Registry,²⁴ and the Brisbane Adolescent Twin Study (BATS), details of which have been published previously.^{25,26} We invited all of the twins and their nontwin siblings to participate in an extensive eye examination and to complete a detailed questionnaire providing relevant sociodemographic and medical information. Participants from both study sites were examined by the same research team after the same protocol.

The study was approved by the ethics committees of the Royal Victorian Eye and Ear Hospital, the Royal Hobart Hospital, and the Queensland Institute of Medical Research, as well as the Australian Twin Registry, and adhered to the tenets of the Declaration of Helsinki. Written informed consent, including consent for genetic analyses, was obtained from all of the participants or their legal guardians, with the participants' assent before examination.

There were 2210 participants from 1040 families in the total sample (1021 from TEST and 1189 from BATS). Of these, 2091 persons had retinal vascular caliber data for both right and left eyes, and an additional 51 individuals had either right or left eye retinal vascular caliber data, composed of 374 monozygotic (MZ) and 536 dizygotic (DZ; 256 opposite-sex DZ) twin pairs, as well as 322 participants analyzed as singletons in the heritability analysis. Data were analyzed by study site because of potential differences in population characteristics.

Retinal Photography and Grading

All of the twins and nontwin siblings had 10° stereoscopic optic disc-centered photographs using a Nidek 3-Dx/F fundus camera (Nidek) after dilatation of the pupils with tropicamide 1% or cyclopentolate 1%. After digitalization of photographs, retinal vascular caliber was measured with computer-assisted software (IVAN, University of Wisconsin) according to a standardized protocol.²⁷ Two trained graders, masked to participant characteristics, performed the vessel measurements on the optic disc-centered image for both eyes for all of the participants. The largest 6 arterioles and venules coursing through a zone between half to 1 disc diameter from the optic disc margin were measured. Images were considered ungradable if quality was poor or if the largest 6 vessels could not be measured. Estimates were summarized as the central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE), representing the average diameter of arterioles and venules of the eye, respectively, using a revised Knudtson-Parr-Hubbard formula.²⁸ Reliability of the retinal vessel measurement has been published elsewhere.²⁵ The intragrader variation was assessed in 67 randomly selected retinal photographs. Intragrader intraclass correlation coefficient was 0.95 for CRAE and 0.99 for CRVE, intergrader reliability was assessed in 52 randomly selected retinal images, and interclass correlation coefficient was 0.93 for CRAE and 0.98 for CRVE.

Zygoty Testing and Genotyping Information

Genomic DNA was extracted from either buccal swabs or venous blood samples. Zygoty in twins of the same sex was confirmed by genotyping ≤ 12 high polymorphism short tandem repeat (STR) markers.²⁹

STR Genome Scan

The genome scan for the BATS was performed at 3 different laboratories: the Australian Genome Research Facility (Melbourne, Australia), Center for Inherited Disease Research (Baltimore, MD), and the Marshfield Mammalian Genotyping Service (Marshfield, WI). Preliminary data of the genome scan have been described in a previous analysis.³⁰ In summary, 644 families composed of 2756 individuals, including 1199 parents, had whole genome scans in the BATS (an average of 410 microsatellite markers from the Australian Genome Research Facility, 386 markers from the Center for Inherited Disease Research, and 394 markers from the Marshfield Mammalian Genotyping Service). After passing our quality-control standards, there were 1190 microsatellite markers (900 unique markers) included in the final analysis; the genome-wide average marker distance for sibling pairs both typed was 8.31 centimorgans (cM).

Currently, there are no genome-wide scans performed for the TEST cohort.

Single Nucleotide Polymorphisms Genome Scan

The 100K single nucleotide polymorphism (SNP) data were from the Affymetrix 100K SNP chip marker set (109 511 SNPs) genotyped at the Australian Genome Research Facility. All of the DNA of children for 169 families of BATS was genotyped. Genotyping data (50K Array Xba 240) were also available for both parents in 104 families and 1 parent in 7 families, with 58 families having no parent genome information. We used the program GRR (Graphical Representation of Relationships) to confirm the expected family relationship between individuals.³¹ After removing $\approx 5\%$ of the SNPs according to Hardy-Weinberg equilibrium testing (dropping cutoff $P < 0.001$), there were 6.1% of the SNPs with minor allele frequency (MAF) $< 1\%$ in the SNP scan set. In total, there were 109 511 SNPs in the scan set, 102 802 of the SNPs (93.9%) were common, and SNPs with minor allele frequency $< 5\%$ were excluded from the SNP linkage analysis. Among these families, 192 individuals from 90 families had retinal vascular caliber measurements.

Combined STR and SNP Linkage Set

A smaller linkage data set (15K) was created from the 100K SNP scan data set by restricting the minimum distance between adjacent SNPs to 0.1 cM. The additional 169 families with genome scans of SNPs data were then combined with the families with STR to increase sample size and genotyping information and, thus, to increase statistical power to detect QTL.

The pedigree information for the combined linkage set (all STR markers and linkage SNP set) was from 811 families consisting of 1409 parents and 1946 children. Taken together, there were 17 006 markers (STR and SNP), and the average marker distance was 0.21 cM, with the total coverage of 3570 cM.

Definition of Other Variables

In brief, all of the twins and siblings completed a detailed questionnaire, including demographic information and medical history, and underwent clinical and eye examinations.³² Age was defined as age at eye examination. The majority of the participants had height and weight measured at the time of the eye examination (1502 [71.8%] of 2091 persons), and the rest of the anthropometry data were derived from previous records in the BATS (71 persons). Twins from the BATS also had additional previous extra data collected, including blood pressure, total cholesterol, high-density lipoprotein (HDL) cholesterol, fasting blood glucose, and so forth, and, on average, 50% of these measurements were collected 4 years before the eye examination. Mean arterial blood pressure (MABP) was calculated as two thirds of the diastolic blood pressure plus one-third of the systolic value. Body mass index (BMI) was calculated as kilograms per meter squared.

Statistical Analysis

Both right and left eye retinal arteriolar caliber and venular caliber followed approximately normal distribution and were analyzed as quantitative traits. We compared means and variance differences of covariates, such as age, MABP, BMI, total cholesterol, fasting blood glucose, and retinal vascular caliber by sex, among different samples.

Variance component modeling was performed using the software package Mx³³ to estimate the proportion of variance explained by genetic and environmental effects. Total residual phenotypic variance of retinal arteriolar and venular calibers was partitioned into additive (A) and dominant (D) genetic variances and common (C) and unique environment (E) variances for the right and left eyes in bivariate path models. Parameter estimates for right and left eyes were tested and set to equal in all of the models.

Age, sex, and BMI were used as covariates in the model fitting for the TEST sample. We replaced the missing covariate data using the mean value of the covariate; therefore, there was no compromise with regard to the sample size. Although those missing covariates

Table 1. Intrapair Correlation of the Retinal Vascular Caliber Estimated Using Mx

Traits	Study Sample	Zygoty	No. of Twin Pairs	Intrapair Correlation (95% CI)
Retinal arteriolar caliber	TEST	MZ	175	0.60 (0.53 to 0.66)
		DZ	252	0.34 (0.25 to 0.42)
	BATS	MZ	180	0.74 (0.68 to 0.78)
		DZ	261	0.42 (0.32 to 0.51)
Retinal venular caliber	TEST	MZ	175	0.63 (0.57 to 0.69)
		DZ	252	0.28 (0.19 to 0.37)
	BATS	MZ	180	0.75 (0.70 to 0.79)
		DZ	261	0.35 (0.24 to 0.45)

replaced by the mean value will not contribute to the covariate effects, for this analysis conducted in a relatively young population where the genetic contribution may be stronger and environmental factors may have less influence (eg, BATS), this impact may be minimal. Replacement of the missing values also has additional advantages, such as allowing for a comparison with work published previously. We included MABP, fasting glucose, cholesterol, and HDL cholesterol as additional covariates in model fitting for the BATS. The heritability was then estimated as the proportion of genetic variance that was attributable to the total residual phenotypic variance.

In our study sample, the second sets of twins, triplets, and nontwin siblings in some families were treated as singletons to avoid complexity of the within-family relationship; therefore, they did not contribute to the covariance in the model fitting. However, these participants have still contributed to the estimation of the mean and variance and, thus, stabilized the estimates of twin correlations for retinal vascular caliber.

We performed variance component linkage analysis for the mean of the left and right eye retinal vascular caliber measurements using the statistical package Merlin.³⁴ Parameter estimates were obtained by maximum likelihood methods. We also performed a gene-dropping simulation using the Merlin program. After 1000 simulations, logarithm of the odds (LOD) scores of 1.69 and 2.04 were found to be suggestive for genome-wide linkage of retinal arteriolar and venular calibers, respectively.³⁵ Also from the simulation, significant genome-wide linkage LOD scores were 3.30 and 3.99 for retinal arteriolar and venular calibers. This is higher than the conventional significant linkage LOD score threshold of 3.0 because of pedigree size, data distribution, and genotype information.

Power calculation was performed for a continuous trait according to the method as described by Purcell et al.³⁶ Our sample had >90% power to detect a linked QTL accounting for $\geq 15\%$ of the trait variation in retinal vascular caliber.

Results

There were 2091 participants from 963 families with retinal vessel measurements for both eyes. Please see the online Data Supplement (<http://hyper.ahajournals.org>) for Table S1, which displays the descriptive statistics of the phenotypes and covariates for males and females by study site. There were no significant differences in the mean and variance of the retinal vascular caliber between males and females (except for the variance of the right eye retinal venular caliber in TEST). There was also no significant difference in the mean and variance of the retinal vascular caliber between MZ and DZ twins (except for the mean retinal venular caliber of the right eye in BATS; data not shown).

There were no significant differences in the overall means or variances of retinal arteriolar caliber and venular caliber between twins and their nontwin siblings for both eyes (eg, mean retinal arteriolar caliber of right eye for twins 164.85

[95% CI: 164.21 to 165.48] and for siblings 163.49 [95% CI: 161.48 to 165.50]; $P=0.21$; data not shown).

Both MABP and BMI were significantly correlated with retinal arteriolar caliber (correlation coefficients: -0.21 and -0.18 for the right eye, -0.15 and -0.23 for the left eye, and -0.20 and -0.23 for the mean of right and left eye measurements, respectively; all $P<0.05$) but not correlated with retinal venular caliber. However, neither arteriolar nor venular calibers were significantly correlated with height or other covariates, including sex, cholesterol, HDL cholesterol, and fasting glucose (correlation coefficient ranged from -0.064 to 0.092 ; $P>0.05$).

In both TEST and BATS cohorts, age was inversely associated with retinal arteriolar and venular caliber (all $P<0.007$ for CRAE and CRVE; data not shown). In the BATS cohort, MABP only had a marginal effect on retinal arteriolar caliber ($P=0.02$; data not shown). Other covariates, including sex, BMI, total cholesterol, HDL cholesterol, and glucose, had little effect on retinal arteriolar or venular caliber.

The correlation of retinal arteriolar caliber for the right and left eyes within twin 1 or twin 2 (within twin cross trait) was 0.69 in the TEST sample (0.90 for the BATS). For venular caliber, the correlation between the right and left eyes within twin 1 or twin 2 was 0.72 in the TEST (0.88 for the BATS). The phenotypic correlation between retinal arteriolar and venular calibers within twin 1 or twin 2 was 0.54 for the combined sample (0.58 for the TEST and 0.51 for the BATS).

Heritability Analysis

The intrapair correlations of both retinal arteriolar and venular calibers were significantly higher among MZ twins than that among DZ twins (Table 1), suggesting that a genetic effect may be involved, and, subsequently, bivariate general ACE and ADE models for the retinal arteriolar and venular calibers of each twin's right and left eyes were fitted, respectively (Table 2). The most parsimonious model for retinal arteriolar caliber was the AE model (Figure 1), in which an additive genetic variance (A) accounted for 59.4% (95% CI: 53.2% to 64.7%) of variance in the arteriolar caliber of each eye for the TEST sample (56.5% [95% CI: 50.1% to 61.9%] for BATS). Individual environmental factors (E) explained 9.3% (95% CI: 4.8% to 14.9%) of the variance for each eye in this sample (11.4% [95% CI: 6.5% to 17.4%] for BATS). The remaining 31.3% (95% CI: 27.9% to 35.0%) variance represented environmental influence specific (e) to

Table 2. ACE/ADE Model Fitting Results From Mx for Retinal Vascular Caliber

Trait	Sample	Models	A	C	D	E	e	-2LL	Degrees of Freedom	AIC
Retinal arteriolar caliber	TEST	ADE	59.4	—	0.0	9.3	31.3	4342.5	1877	588.5
		ACE	49.3	9.5	—	10.1	31.1	4341.4	1877	587.4
		AE*	59.4	—	—	9.3	31.3	4342.5	1878	586.5
	BATS	ADE	56.5	—	0.0	11.4	32.1	5659.0	2340	979.0
		ACE	56.5	0.0	—	11.4	32.1	5658.8	2340	978.8
		AE*	56.5	—	—	11.4	32.1	5659.0	2341	977.0
Retinal venular caliber	TEST	ADE	58.6	—	3.2	10.0	28.2	4447.1	1877	693.1
		ACE	61.7	0.0	—	10.1	28.2	4447.2	1877	693.2
		AE*	61.7	—	—	10.1	28.2	4447.2	1878	691.2
	BATS	ADE	45.0	—	19.6	4.3	31.1	5594.3	2340	914.3
		ACE	64.2	0.0	—	4.9	30.9	5595.4	2340	915.4
		AE*	64.2	—	—	4.9	30.9	5595.4	2341	913.4

A indicates additive genetics; C, common environment; D, dominant genetics; E, unique environment; e, specific component; AIC, Akaike's Information Criterion; —, no data. The comparisons among ADE, ACE, and AE models were based on the final saturated model, in which some parameters were constrained to equal, and those specific latent effects, such as a, c, or d, were dropped. Values used in the table for A(C|D)Ee were standardized proportion of variances (%).

*Data show the best-fit model.

each eye (32.1% [95% CI: 29.1% to 35.5%] for BATS), which included measurement error.

The most parsimonious model for retinal venular caliber was the AE model (Figure 1); additive genetic variance accounted for 61.7% (95% CI: 55.6% to 67.0%) of the variance in the retinal venular caliber of each eye for the TEST sample (64.2% [95% CI: 58.7% to 68.8%] for BATS). Individual environmental factors explained 10.1% (95% CI: 5.6% to 15.6%) of the variance for each eye in this sample (4.9% [95% CI: 1.0% to 9.8%] for BATS). The remaining 28.2% (95% CI: 25.0% to 31.6%) variance represented environmental influence specific to each eye (30.9% [95% CI: 27.9% to 34.2%] for BATS). For the BATS, the model fitting was not improved by adding additional covariates (MABP, fasting glucose, cholesterol, or HDL cholesterol), and these additional covariates were dropped from the final model (data not shown).

Sex-limitation modeling was performed using male and female parameters for the best-fitting model (AEe) and showed no evidence of sex differences for variance compo-

nents in the retinal arteriolar caliber. However, venular caliber showed small differences in variance components between sexes; in the combined TEST and BATS samples, the genetic effect in males (63.9%) was only slightly higher than in females (60.4%), whereas the individual environmental factor affecting both eyes was slightly higher in females (9.3%) than in males (6.4%).

Genome-Wide Linkage Analysis

Please see Table S2, available online at <http://hyper.ahajournals.org>, which shows the breakdown of participants who contributed linkage data. There were 836 individuals from 381 families, consisting of 511 quasi-independent sibling pairs from the BATS sample for linkage analysis.

The results of the linkage analysis for retinal vascular caliber are displayed in Figures 2 and 3, respectively. No genome-wide significant LOD score was detected for either the retinal arteriolar caliber or venular caliber. Two multipoint peaks for the retinal arteriolar caliber were observed on chromosomes 3p12.3 and 8p23.1, indicating a suggestive (or weaker) linkage (Table 3 and Figure 2). The highest multipoint peak was an LOD score of 2.24 on chromosome 8p23.1 (genome-wide $P=7.0 \times 10^{-4}$, after adjustment for age, sex, MABP, BMI, fasting blood glucose, total cholesterol, and HDL cholesterol). Although these covariates had minimal effect on the heritability analyses, they were used in the linkage analysis for 2 reasons: the first was to match findings from other studies in the literature for comparison and the second was to include those covariates to improve the distribution of traits.

We also identified 2 suggestive loci for the retinal venular caliber on chromosomes 2p14 and 9q21.13 (Table 3 and Figure 3). The largest multipoint LOD score was 2.69 on chromosome 2p14 (genome-wide $P=2.0 \times 10^{-4}$, after adjustment for age, sex, MABP, and relevant covariates). We then examined for individual family contribution to the total LOD score and found that 1 family contributed the highest LOD score of 1.72. This family contribution was reasonable given

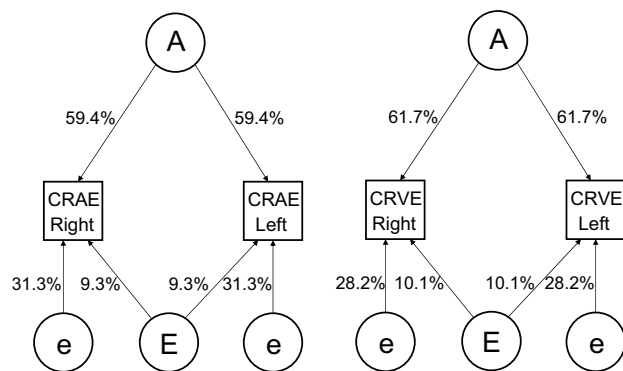


Figure 1. Path diagram illustrating parameter specification in the Bivariate AE model: additive genetic component (A), unique environment component (E), and specific (e) components of variance for CRAE and CRVE of both eyes in each twin of the TEST sample.

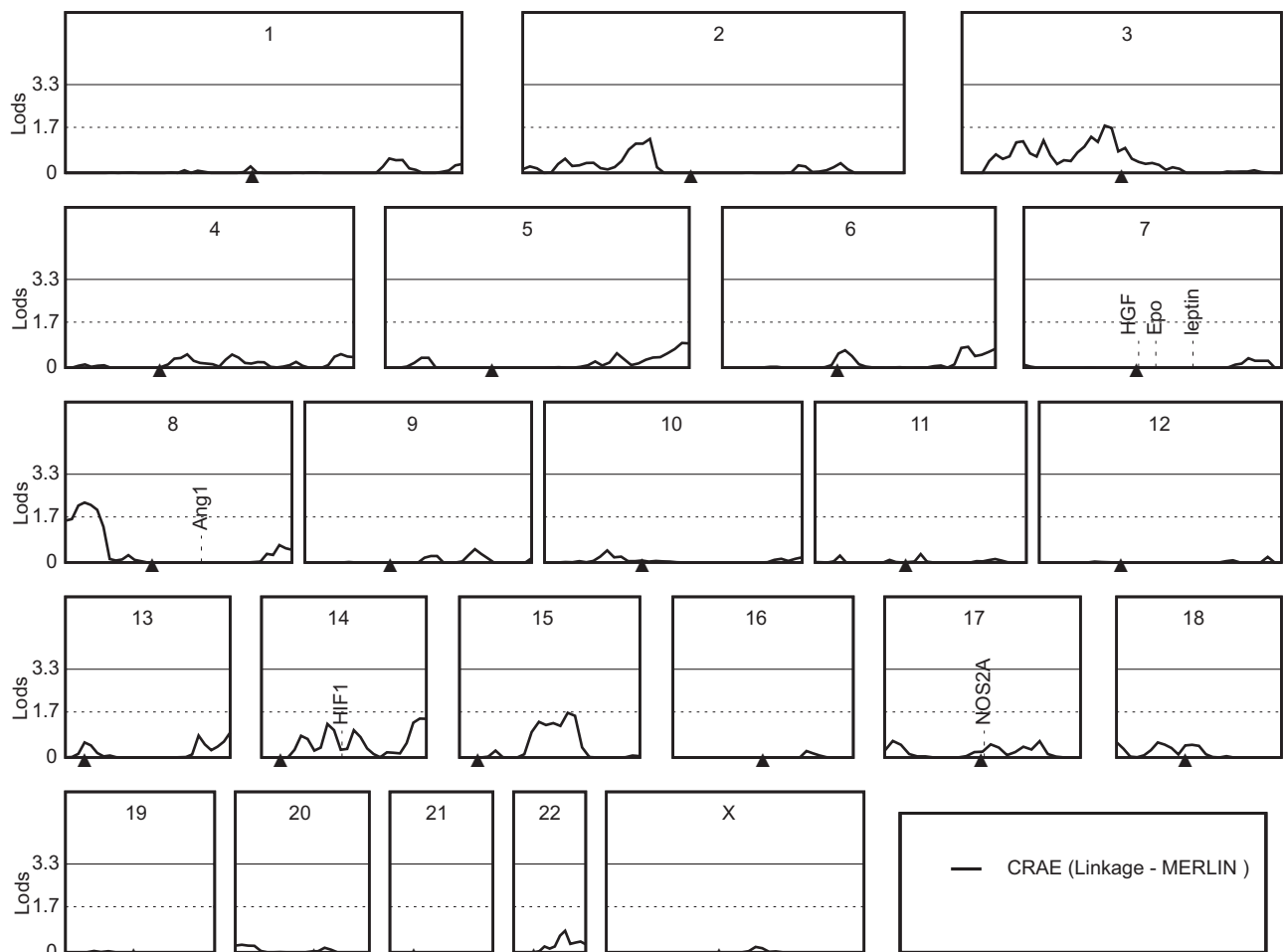


Figure 2. Genome-wide linkage analysis for retinal arteriolar caliber. Chromosome positions are displayed on the x-axis, and the y-axis displays the strength of evidence for linkage (LOD).

that it was likely to have occurred because they were the most informative family, with 4 siblings and both parents providing genome scans (10 independent-quasi sibling pairs), rather than because they were an outlying family.

Discussion

In the study population composed of >2000 twins and their siblings, we demonstrated that retinal arteriolar and venular calibers were heritable traits with common genetic variation accounting for $\approx 60\%$ of the normal variation in the twin population. We used the bivariate path model to partition the residual phenotypic variance, taking into account the phenotypes of 4 eyes and the latent variables as well.³⁷ In a subset of this population, the genome-wide linkage analysis identified several suggestive linkage signals for both retinal arteriolar and venular calibers. The highest multipoint peak for retinal arteriolar caliber was on chromosome 8p23.1 with an LOD score of 2.24, and the largest multipoint LOD score for the retinal venular caliber was 2.69 on chromosome 2p14.

Our estimation of heritability of the retinal arteriolar and venular calibers was similar to but lower than that reported in a previous twin study from the Danish Twin Registry, which showed that heritabilities of retinal arteriolar and venular calibers were 70% (95% CI: 54% to 80%) and 83% (95% CI:

73% to 89%), respectively.²⁰ The small difference in heritability estimation may be a reflection of the subtly different genetic composition of the study populations but is more likely attributed to differences between centers in measuring the retinal caliber. We also found that the covariates (MABP, fasting blood glucose, total cholesterol, and HDL cholesterol) had limited effects on the retinal vascular caliber in the heritability estimation, which is consistent with the Danish Twin Study.²⁰ The estimation of heritability of the retinal arteriolar and venular calibers was similar between the TEST and the BATS, indicating that the results from this study may be largely generalizable to other white twin populations. Furthermore, there were no significant differences in the mean and variance of retinal arteriolar and venular calibers between twins and their nontwin siblings, which increase the confidence in the generalizability of the findings of twin studies to the Australian general population.

We found no evidence of significant linkage regions harboring genetic variants that influence the variation in the retinal vascular caliber. Although there were a number of multipoint peaks at a suggestive linkage level, none of them replicated the linkage regions found in the earlier linkage analysis from the Beaver Dam Eye Study.²¹ Retinal arteriolar and venular calibers showed no shared or overlapped sugges-

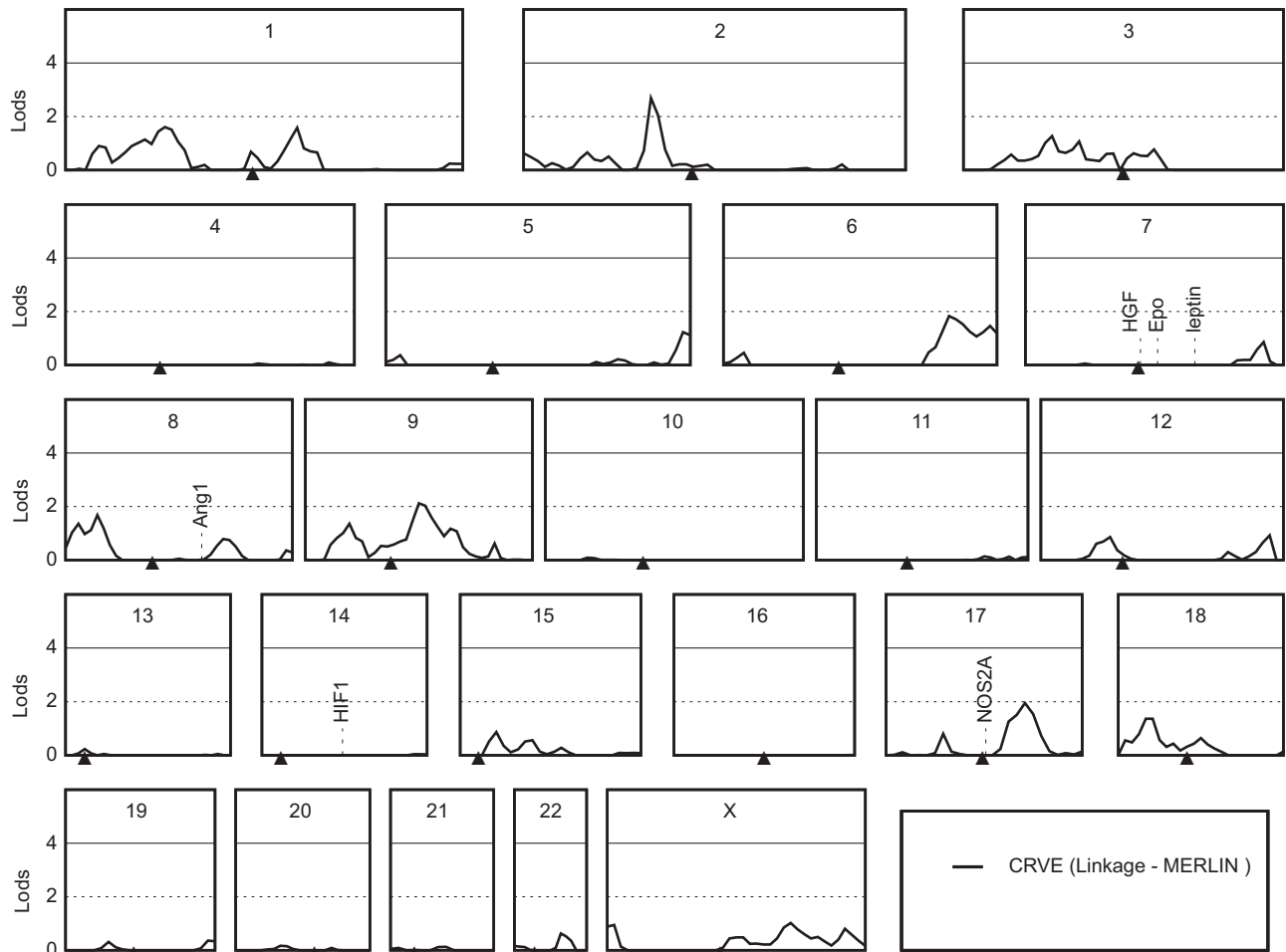


Figure 3. Genome-wide linkage analysis for the retinal venular caliber. Chromosome positions are displayed on the x-axis, and the y-axis displays the strength of evidence for linkage (LOD).

tive linkage regions and were linked to distinct suggestive linkage regions on different chromosomes, which support the notion that the determinants of the variation of arterioles and venules may operate at the genetic level. Interestingly, one strong linkage signal reported in the Beaver Dam Eye Study was at chromosome 3q28 and was only significant for the covariates-adjusted retinal arteriolar caliber. The contrasting finding between the current study and the Beaver Dam Eye Study may be because of the different study population, and some of the identified linkage signals in the previous study may partly reflect the strong covariate effect (eg, linkage peak on chromosome 3q28 for the retinal arteriolar caliber).³⁸

Clearly, it underscores the need for future linkage studies on the retinal vascular caliber.

It is noteworthy that there is a substantial phenotypic correlation between retinal arteriolar and venular calibers. Although we were unable to detect putative shared loci linked to both the retinal arteriolar and venular calibers in the current study, multivariate analyses for venular and arteriolar calibers using both right and left eye information to explore their genetic and environmental overlaps will be the next important step. We also observed a significant correlation of the retinal arteriolar caliber with MABP, which is in line with previous reports from the general population^{1,2}; a bivariate analysis

Table 3. Genome Scan Multipoint Linkage Results for Retinal Vacular Caliber (Mean) With Suggestive LOD Scores

Trait	Genomic Region	Position (cM)	Closest Marker	LOD*	<i>P</i> †
Retinal arteriolar caliber	3p12.3	107.43	D3S3681	1.77	2.0×10^{-3}
	8p23.1	16.35	D8S277	2.24	7.0×10^{-4}
Retinal venular caliber	2p14	91.32	D2S1779	2.69	2.0×10^{-4}
	9q21.13	90.23	D9S175	2.12	9.0×10^{-4}

*Data show the residual mean retinal arteriolar and venular calibers of right and left eyes, after adjusting for age, sex, body mass index, MABP, fasting glucose, cholesterol, and HDL cholesterol.

†Data show multipoint probability.

looking at common genes for the retinal arteriolar caliber and blood pressure in the future will provide additional insights into the shared genetic and environmental factors underlying the covariation. These findings add greater applicable credence for our current study findings to the general population.

There are multiple strengths of this study. These include the population-based sample, well-defined and reproducible measures of the retinal vascular caliber, and the recruitment of nontwin siblings to increase the power of the linkage study and to allow for testing of the representativeness of the twin sample. A number of limitations should be considered for results interpretation. The genome-wide linkage analyses were performed in a subsample of the Brisbane twins. Larger sample sets and additional evidence are required to stabilize the point estimation for QTL mapping, generating replicable and confident results. In addition, some covariate measures (eg, blood pressure) were collected several years before the eye examination, and relevant covariate information was partially missing because the retinal vascular caliber was not the primary study parameter, and its related covariates were, therefore, not part of the original data collection. However, the covariate effect on the genetic analysis in this study is relatively small, and an important covariate (eg, age) was available for all of the participants.

Perspectives

In this large twin study, we demonstrated a strong genetic effect for the variation in retinal arteriolar and venular calibers. However, among the small subset of genotyped individuals, we found little evidence of a strong linkage for both traits in the genome-wide scan. Larger sample sizes and additional evidence are required to stabilize the point estimation for QTL mapping and to generate more confident results.

Sources of Funding

The genome scans were supported by the Australian National Health and Medical Research Council Program in Medical Genomics (NHMRC-219178 to N.G.M. and G.W.M.) and the Center for Inherited Disease Research at Johns Hopkins University (to N.G.M.). The Center for Inherited Disease Research is fully funded through a federal contract from the National Institutes of Health to the Johns Hopkins University (contract No. N01-HG-65403). The research reported in this article was supported by Ophthalmic Research Institute of Australia (to D.A.M., C.S., and J.B.R.), the Foundation for Children Australia (to T.Y.W., D.A.M., and C.S.), the National Health and Medical Research Council, Canberra Australia (project grant 350415 to D.A.M.), the Clifford Craig Medical Research Trust, National Institutes of Health, National Eye Institute (RO1 EY018246-01, to T.L.Y., D.A.M., C.J.H., and N.G.M.). C.S. is the recipient of a National Health and Medical Research Council Public Health Postgraduate Scholarship. D.A.M. is the recipient of the Pfizer Australia Senior Research Fellowship.

Disclosures

None.

References

1. Sun C, Wang JJ, Mackey DA, Wong TY. Retinal vascular caliber: systemic, environmental, and genetic associations. *Surv Ophthalmol*. 2009;54:74–95.
2. Wong TY, Mitchell P. The eye in hypertension. *Lancet*. 2007;369:425–435.

3. Wong TY, Klein R, Klein BE, Tielsch JM, Hubbard L, Nieto FJ. Retinal microvascular abnormalities and their relationship with hypertension, cardiovascular disease, and mortality. *Surv Ophthalmol*. 2001;46:59–80.
4. Wong TY, Mitchell P. Hypertensive retinopathy. *N Engl J Med*. 2004;351:2310–2317.
5. Nguyen TT, Wong TY. Retinal vascular manifestations of metabolic disorders. *Trends Endocrinol Metab*. 2006;17:262–268.
6. Wong TY. Is retinal photography useful in the measurement of stroke risk? *Lancet Neurol*. 2004;3:179–183.
7. Wong TY, Klein R, Couper DJ, Cooper LS, Shahar E, Hubbard LD, Wofford MR, Sharrett AR. Retinal microvascular abnormalities and incident stroke: the Atherosclerosis Risk in Communities Study. *Lancet*. 2001;358:1134–1140.
8. Wong TY, Klein R, Sharrett AR, Duncan BB, Couper DJ, Klein BE, Hubbard LD, Nieto FJ. Retinal arteriolar diameter and risk for hypertension. *Ann Intern Med*. 2004;140:248–255.
9. Wong TY, Klein R, Sharrett AR, Duncan BB, Couper DJ, Tielsch JM, Klein BE, Hubbard LD. Retinal arteriolar narrowing and risk of coronary heart disease in men and women: the Atherosclerosis Risk in Communities Study. *JAMA*. 2002;287:1153–1159.
10. Wong TY, Klein R, Sharrett AR, Schmidt MI, Pankow JS, Couper DJ, Klein BE, Hubbard LD, Duncan BB. Retinal arteriolar narrowing and risk of diabetes mellitus in middle-aged persons. *JAMA*. 2002;287:2528–2533.
11. Leung H, Wang JJ, Rohtchina E, Wong TY, Klein R, Mitchell P. Impact of current and past blood pressure on retinal arteriolar diameter in an older population. *J Hypertens*. 2004;22:1543–1549.
12. Mitchell P, Cheung N, de Haseth K, Taylor B, Rohtchina E, Islam FM, Wang JJ, Saw SM, Wong TY. Blood pressure and retinal arteriolar narrowing in children. *Hypertension*. 2007;49:1156–1162.
13. Wong TY, Kamineni A, Klein R, Sharrett AR, Klein BE, Siscovick DS, Cushman M, Duncan BB. Quantitative retinal venular caliber and risk of cardiovascular disease in older persons: the Cardiovascular Health Study. *Arch Intern Med*. 2006;166:2388–2394.
14. Ikram MK, de Jong FJ, Bos MJ, Vingerling JR, Hofman A, Koudstaal PJ, de Jong PT, Breteler MM. Retinal vessel diameters and risk of stroke: the Rotterdam Study. *Neurology*. 2006;66:1339–1343.
15. Wong TY, Islam FM, Klein R, Klein BE, Cotch MF, Castro C, Sharrett AR, Shahar E. Retinal vascular caliber, cardiovascular risk factors, and inflammation: the multi-ethnic study of atherosclerosis (MESA). *Invest Ophthalmol Vis Sci*. 2006;47:2341–2350.
16. Klein R, Klein BE, Knudtson MD, Wong TY, Tsai MY. Are inflammatory factors related to retinal vessel caliber? The Beaver Dam Eye Study. *Arch Ophthalmol*. 2006;124:87–94.
17. Leung H, Wang JJ, Rohtchina E, Wong TY, Klein R, Mitchell P. Dyslipidaemia and microvascular disease in the retina. *Eye*. 2005;19:861–868.
18. Wong TY, Duncan BB, Golden SH, Klein R, Couper DJ, Klein BE, Hubbard LD, Sharrett AR, Schmidt MI. Associations between the metabolic syndrome and retinal microvascular signs: the Atherosclerosis Risk in Communities Study. *Invest Ophthalmol Vis Sci*. 2004;45:2949–2954.
19. Lee KE, Klein BE, Klein R, Knudtson MD. Familial aggregation of retinal vessel caliber in the Beaver Dam Eye Study. *Invest Ophthalmol Vis Sci*. 2004;45:3929–3933.
20. Taarnhoj NC, Larsen M, Sander B, Kyvik KO, Kessel L, Hougaard JL, Sorensen TI. Heritability of retinal vessel diameters and blood pressure: a twin study. *Invest Ophthalmol Vis Sci*. 2006;47:3539–3544.
21. Xing C, Klein BE, Klein R, Jun G, Lee KE, Iyengar SK. Genome-wide linkage study of retinal vessel diameters in the Beaver Dam Eye Study. *Hypertension*. 2006;47:797–802.
22. Sun C, Wang JJ, Islam FM, Heckbert SR, Klein R, Siscovick DS, Klein BE, Wong TY. Hypertension genes and retinal vascular calibre: the Cardiovascular Health Study. *J Hum Hypertens*. 2009;23:578–584.
23. Wang JJ, Wong TY. Genetic determinants of retinal vascular caliber: additional insights into hypertension pathogenesis. *Hypertension*. 2006;47:644–645.
24. Hopper JL. The Australian Twin Registry. *Twin Res*. 2002;5:329–336.
25. Sun C, Ponsonby AL, Wong TY, Brown SA, Kearns LS, Cochrane J, MacKinnon JR, Ruddle JB, Hewitt AW, Liew G, Terrence Dwyer, Scurrah K, Mackey DA. Effect of birth parameters on retinal vascular caliber: the Twins Eye Study in Tasmania. *Hypertension*. 2009;53:487–493.
26. Wright MJ, Martin NG. Brisbane adolescent twin study: outline of study methods and research projects. *Aust J Psychol*. 2004;56:65–78.

27. Wong TY, Knudtson MD, Klein R, Klein BE, Meuer SM, Hubbard LD. Computer-assisted measurement of retinal vessel diameters in the Beaver Dam Eye Study: methodology, correlation between eyes, and effect of refractive errors. *Ophthalmology*. 2004;111:1183–1190.
28. Knudtson MD, Lee KE, Hubbard LD, Wong TY, Klein R, Klein BE. Revised formulas for summarizing retinal vessel diameters. *Curr Eye Res*. 2003;27:143–149.
29. Spitz E, Moutier R, Reed T, Busnel MC, Marchaland C, Roubertoux PL, Carlier M. Comparative diagnoses of twin zygosity by SSLP variant analysis, questionnaire, and dermatoglyphic analysis. *Behav Genet*. 1996; 26:55–63.
30. Zhu G, Evans DM, Duffy DL, Montgomery GW, Medland SE, Gillespie NA, Ewen KR, Jewell M, Liew YW, Hayward NK, Sturm RA, Trent JM, Martin NG. A genome scan for eye color in 502 twin families: most variation is due to a QTL on chromosome 15q. *Twin Res*. 2004;7:197–210.
31. Abecasis GR, Cherny SS, Cookson WO, Cardon LR. GRR: graphical representation of relationship errors. *Bioinformatics*. 2001;17:742–743.
32. Toh T, Liew SH, MacKinnon JR, Hewitt AW, Poulsen JL, Spector TD, Gilbert CE, Craig JE, Hammond CJ, Mackey DA. Central corneal thickness is highly heritable: the Twin Eye Studies. *Invest Ophthalmol Vis Sci*. 2005;46:3718–3722.
33. Neale MC, Boker SM, Xie G, Maes HH. Mx: Statistical Modeling [computer program]. 6th Version. Richmond, VA: Department of Psychiatry. 2002.
34. Abecasis GR, Cherny SS, Cookson WO, Cardon LR. Merlin—rapid analysis of dense genetic maps using sparse gene flow trees. *Nat Genet*. 2002;30:97–101.
35. Kong A, Gudbjartsson DF, Sainz J, Jonsdottir GM, Gudjonsson SA, Richardsson B, Sigurdardottir S, Barnard J, Hallbeck B, Masson G, Shlien A, Palsson ST, Frigge ML, Thorgeirsson TE, Gulcher JR, Stefansson K. A high-resolution recombination map of the human genome. *Nat Genet*. 2002;31:241–247.
36. Purcell S, Cherny SS, Sham PC. Genetic power calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics*. 2003;19:149–150.
37. Zhu G, Hewitt AW, Ruddle JB, Kearns LS, Brown SA, Mackinnon JR, Chen CY, Hammond CJ, Craig JE, Montgomery GW, Martin NG, Mackey DA. Genetic dissection of myopia: evidence for linkage of ocular axial length to chromosome 5q. *Ophthalmology*. 2008;115:1053–1057.e2.
38. Hopper JL. Variance components for statistical genetics: applications in medical research to characteristics related to human diseases and health. *Stat Methods Med Res*. 1993;2:199–223.

Running Title: Genetics of Retinal Vascular Caliber

Quantitative Genetic Analysis of Retinal Vascular Caliber: the Australian Twins

Eye Study

Cong Sun, MD, MPH¹; Gu Zhu, MD, MPH²; Tien Y. Wong, MD, PhD^{1,3}; Alex W. Hewitt, MBBS¹; Jonathan B. Ruddle, MBBS¹; Lauren Hodgson, BSc¹; Grant W. Montgomery, PhD²; Terri L. Young, MD, PhD⁴; Christopher J. Hammond, MD, PhD⁵; Jamie E. Craig, MBBS, DPhil⁶; Nicholas G. Martin, PhD²; Mingguang He, MD, PhD⁷; David A. Mackey, MD^{1,8}

¹Centre for Eye Research Australia, University of Melbourne, Melbourne, Australia

²Queensland Institute of Medical Research, Brisbane, Australia

³Singapore Eye Research Institute, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

⁴Duke Eye Centre, Duke University Medical Centre, Duke University, Durham, NC, USA

⁵Twin Research and Genetic Epidemiology Unit, St. Thomas's Hospital, London, UK

⁶National Health and Medical Research Council Centre for Clinical Eye Research, Department of Ophthalmology, Flinders University, Flinders Medical Centre, Adelaide, Australia

⁷State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, China

⁸Discipline of Medicine, University of Tasmania, Australia

Correspondence to: A/Prof David A. Mackey
Centre for Eye Research Australia
University of Melbourne, Department of Ophthalmology
Royal Victorian Eye and Ear Hospital
1/32 Gisborne St
EAST MELBOURNE, VIC 3002
Tel: +61 3 99298713, Fax: +61 3 99298164
Email: D.Mackey@utas.edu.au

Manuscript word count: 5982

Number of Figures: 3

Online Supplement Tables

Table S1. Participant Characteristics for Phenotypes and Covariates, By Sex and Study Sample

Study Sample	Female		Male		P value*
TEST	N	Mean (SD)	N	Mean (SD)	
CRAE_R	537	165.39 (15.11)	389	165.85 (15.17)	0.65 (0.94)
CRAE_L	537	164.48 (15.44)	389	164.19 (15.34)	0.78 (0.89)
CRVE_R	537	247.93 (21.84)	389	247.58 (19.59)	0.80 (0.02)
CRVE_L	537	247.54 (21.91)	389	246.14 (20.09)	0.31 (0.07)
Age	537	22.58 (17.35)	389	19.68 (17.17)	0.01 (0.82)
BMI	261	24.80 (5.02)	154	24.36 (4.59)	0.36 (0.23)
BATS					
CRAE_R	630	164.56 (14.10)	535	164.16 (13.98)	0.63 (0.93)
CRAE_L	630	165.43 (14.04)	535	164.97 (13.56)	0.57 (0.41)
CRVE_R	630	249.01 (19.57)	535	249.09 (19.45)	0.94 (0.77)
CRVE_L	630	249.97 (18.71)	535	250.93 (18.89)	0.38 (0.90)
Age	630	20.37 (4.20)	535	20.36 (3.87)	0.97 (0.05)
BMI	627	22.25 (4.05)	531	23.09 (3.92)	0.003 (0.44)
Glucose	229	3.66 (1.30)	219	3.57 (1.40)	0.51 (0.26)
MABP	171	81.93 (6.33)	118	84.07 (8.30)	0.02 (0.001)
Total cholesterol	236	3.88 (0.76)	222	3.77 (0.87)	0.18 (0.05)
HDL cholesterol	235	0.99 (0.11)	222	0.99 (0.15)	0.78 (<0.001)

TEST: Twins Eye Study in Tasmania; BATS: Brisbane Adolescent Twin Study; CRAE: central retinal arteriolar equivalent; CRVE: central retinal venular equivalent; SD: standard deviation; HDL: high-density lipoprotein; MABP: mean arterial blood pressure; BMI: body mass index; R: right eye; L: left eye

*P value for comparison of means between males and females, the P value between brackets is for test the differences in standard deviation.

Table S2. Extra Sibling and Parental Genotyped by Zygosity for Retinal Vascular Caliber Linkage Analysis

Zygosity	Twin Pairs	Extra Sibs	Parents Genotyped			Families	Individuals	QISP*
			0	1	2			
MZ	Single	0	5		9	14	14	
		1	3		11	14	28	14
		2	1		1	2	6	6
	Pair	0	2	1	15	18	36	
		1	4	4	18	26	78	26
		2	0	0	5	5	20	15
		Total	15	5	59	79	182	61
DZ	No	1	0	2	2	4	4	
		2	1	0	2	3	6	3
	Single	0	6	8	14	28	28	
		1	0	1	3	4	8	4
		2	0	0	1	1	3	3
	Pair	0	25	41	131	197	394	197
		1	6	5	38	49	147	147
		2	0	5	11	16	64	96
	Total	38	62	202	302	302	654	450
						Total	381	836

MZ: monozygotic; DZ: dizygotic

*Quasi-Independent Sib Pairs= $s(s-1)/2$ for DZ twins and $(s-1)(s-2)/2$ for MZ twins

(where s = total number of sibs)

No: stands for no twin participated in the family