

Familial Aggregation of Retinal Vessel Caliber in the Beaver Dam Eye Study

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PURPOSE. To describe familial correlations of retinal vessel measurements.

METHODS. A standardized examination and interview was administered to a population-based cohort of adults aged 43–86 years. Blood pressure was measured, and family relationships, current smoking status, and photographs of the retina were obtained. Computer-assisted grading was done to determine central retinal arteriole (CRAE) and venule equivalents (CRVE), and the arteriole-to-venule ratio (AVR) was computed. Familial correlations were calculated using FCOR in the SAGE software package. Analysis was done on the right eye measures for 871 sibling, 341 parent–child, 1362 cousin, 554 avuncular, and 887 spousal pairs.

RESULTS. After adjustment for age, gender, mean arterial blood pressure, and current smoking status, the correlations (and 95% confidence interval) between siblings for the CRVE, CRAE, and AVR were 0.23 (0.16, 0.31), 0.20 (0.12, 0.28) and 0.13 (0.05, 0.20), respectively. Parent–child correlations were very similar, and the avuncular correlations were about half as great. The cousin correlations were about half the avuncular correlations. Spousal correlations of 0.03, 0.04, and 0.01 for CRVE, CRAE, and AVR, respectively, were not significantly different from 0.

CONCLUSIONS. Retinal vessel equivalents were more highly correlated between relatives than between unrelated individuals. The relative magnitudes of these correlations were likely the result of shared genes. Because the vessel measurements have been shown to be predictive of cardiovascular and other systemic diseases, understanding the determinants of these familial relationships could have important health benefits. (*Invest Ophthalmol Vis Sci.* 2004;45:3929–3933) DOI:10.1167/iivs.04-0462

Retinal vessels are easily imaged and provide a window to microvascular systems elsewhere in the body. A relationship between retinal vessel characteristics and hypertension has been reported,^{1–5} but until recently has been difficult to assess accurately.^{6–8} Quantification of generalized arteriolar

narrowing using computer-assisted grading has been developed^{9,10} and is highly reproducible.^{9,11,12} Data from a number of studies have shown a relationship of hypertension to a narrowing of retinal arterioles.^{13–16} Reduced arteriolar diameter is also predictive of incident hypertension,¹⁷ stroke,¹⁸ and cardiovascular disease mortality¹⁹ in men and women, and coronary heart disease in women,²⁰ independent of blood pressure and other risk factors.

Risk of hypertension,^{21–24} cardiovascular disease and mortality,^{25–28} and stroke^{29–31} are affected by family history of these disorders. These are complex diseases with multiple genetic and environmental factors influencing their development and manifestations. The familial aggregation observed for cardiovascular disease may be due to concomitant aggregation of these risk factors.²⁶ Because retinal vessel caliber is associated with cardiovascular disease and stroke, understanding the familial determinants of retinal vessel caliber may provide insights into the pathogenesis of these complex diseases. To our knowledge, there have been no studies to date that have evaluated the possibility of genetic influence for retinal vessel caliber.

A first step to investigate the possibility of genetic influences is to examine correlations among various family members. Patterns of correlations that exist between family members, but not between unrelated individuals, provide insight into the existence of common genetic influences. The purpose of these analyses was to examine these familial correlations in the Beaver Dam Eye Study population.

METHODS

The Beaver Dam Eye Study is a population-based cohort study of age-related eye diseases. Procedures have been previously reported.³² Eighty-three percent (4926) of persons who were 43 to 86 years and living in Beaver Dam, WI, participated in the baseline examination from 1988 to 1990. The study adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from each subject, and institutional human experimentation committee approval was granted. As part of this baseline examination, blood pressure was measured using a random-zero sphygmomanometer according to the Hypertension Detection and Follow-up Program protocol,³³ and information was collected about family members, medical history, and lifestyle information, including smoking. After the examination, casual blood specimens were obtained from which blood glucose and glycosylated hemoglobin were assayed.^{34,35} Participants tended to be younger than nonparticipants.³²

During the examination, both eyes were dilated and stereoscopic 30° color fundus photographs, centered on the optic disc, were taken (Diabetic Retinopathy Study Standard Field 1).³⁶ The photographs for the right eyes were converted to digital images by a high-resolution scanner (Nikon LS2000; Nikon Inc., Tokyo, Japan). A grader identified the arterioles and venules, then used a semiautomated procedure to measure the vessel caliber (in microns) within a specified section of the photograph (zone B).⁹ Zone B was the portion of the photograph between circles with 0.5 and 1.0 disc diameters from the optic disc margin.

The measurements for the arterioles were combined into a central retinal artery equivalent (CRAE), while the measurements for the

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TABLE 1. Distribution of Retinal Vessel Equivalents in the Beaver Dam Eye Study, 1988-1990

	Full Population			Persons in Family				
	<i>n</i>	Mean	STD	Min.	Max.	<i>n</i>	Mean	STD
Venule (CRVE)	4231	242.08	22.86	165.1	352.9	3173	242.11	22.70
Arteriole (CRAE)	4241	165.29	15.42	98.1	223.4	3179	165.42	15.40
Ratio (AVR)	4226	0.69	0.06	0.48	0.95	3168	0.69	0.06

CRVE, central retinal venule equivalent; CRAE, central retinal arteriole equivalent; AVR, arteriole-to-venule ratio.

venules were combined into a central retinal vein equivalent (CRVE), using formulas described by Knudtson et al.¹⁰ The formulas were based on a modification of the Parr⁸ and Hubbard⁹ formulas where the six largest vessels were combined successively using the following formulas:

$$\text{Artery: } W = 0.88 * (w_1^2 + w_2^2)^{1/2}$$

$$\text{Vein: } W = 0.95 * (w_1^2 + w_2^2)^{1/2}$$

where W represents the width of the parent vessel, and w_1 and w_2 represent the width of each branching vessel.

Based on information collected at the baseline examination, such as names and residence of siblings, initial family relationships among participants were identified. The family relationships and extended pedigree information were confirmed during follow-up examinations and phone contact of at least one family member. To be a member of a pedigree, an individual needed to be eligible for participation in the Beaver Dam Eye Study and be related to another eligible person through a sibling, parent, child, first cousin, or avuncular (aunt/uncle, niece/nephew) relationship. There were a total of 2783 people in 602 families in the Beaver Dam Eye Study population. Of these 2783 persons, 427 were not examined, and 352 did not have measurable vessels. From the remaining 2004 persons, 160 no longer had a direct relative with data, which reduced the number of family groups from 602 to 441. Among these 441 families, 145 families (containing 332 individuals) were sibling groups only, 106 families (containing 247 individuals) were nuclear family units (at least one parent-child relationship), and the remaining 190 families (1265 individuals) were extended families with at least one avuncular or cousin relationship. Seventy-five percent of these extended families had at least one sibling group (two or more siblings with data), 43% had cousin but no avuncular relationships, 24% had avuncular relationships, and 33% had both cousin and avuncular relationships represented.

In addition to the people already in families, a number of people had a spouse in the study, but were not included in a family because they did not have a child that was part of the study. So that these people would be considered as spousal pairs in the SAGE program, we created children without data values, added 394 individuals who were

married to someone already in a family, and 910 individuals as part of 455 spousal pairs.

Correlations between pairs of relatives were calculated using the FCOR procedure in the computer software program Statistical Analysis for Genetic Epidemiology (S.A.G.E.; Statistical Solutions, Ltd., Cork, Ireland) package.³⁷ The procedure also produces standard errors, which were used to construct 95% confidence intervals around the correlations. A 95% confidence interval that does not include 0 indicates a significant correlation. SAS³⁸ was used to adjust the central retinal equivalents and AVR for age, sex, mean arterial blood pressure, and smoking before analyses. Mean arterial blood pressure was calculated as two-thirds of the diastolic plus one-third of the systolic value. Hypertension was defined as systolic blood pressure ≥ 160 and/or diastolic blood pressure ≥ 95 and/or current use of antihypertensive medication. Diabetes was defined as a previous history of diabetes and/or hyperglycemia. Hyperglycemia was defined as glycosylated hemoglobin > 2 SDs above the mean for the appropriate age and sex group or a casual blood glucose level > 200 mg/dL (1.1 mM/L).

RESULTS

The distribution of the vessel measurements among those in families (including spouse pairs) was similar to the full population of Beaver Dam (Table 1). Sibling correlations for the CRAE and CRVE were 0.20 and 0.23, respectively (Table 2). The sibling correlation for the AVR was 0.13. For all vessel measurements, the parent-child correlations were similar to the sibling correlations. The avuncular correlations were considerably lower for all measures and significantly different from zero for CRVE only. The cousin correlations were lower than the avuncular correlations. The spousal correlations were not different from zero. In general, the correlations for the arterioles and venules were similar and were higher than the correlations for the AVR.

Heritability was estimated by doubling the parent-child correlations and reflects the proportion of the overall population variation that can be attributable to genetic differences. Heritability above 0.30 is generally considered as high. The heritability of the CRAE, CRVE, and the AVR were 0.48, 0.54,

TABLE 2. Familial Correlations of Retinal Vessel Equivalents in the Beaver Dam Eye Study, 1988-1990

Relationship	Number of Pairs	Correlation (95% Confidence Interval)		
		Venule (CRVE)	Arteriole (CRAE)	Ratio (AVR)
Sibling	871	0.23 (0.16, 0.31)	0.20 (0.12, 0.28)	0.13 (0.05, 0.20)
Parent-Child	341	0.24 (0.14, 0.35)	0.27 (0.16, 0.37)	0.16 (0.05, 0.27)
Avuncular	554	0.13 (0.02, 0.24)	0.06 (-0.04, 0.17)	0.07 (-0.02, 0.17)
Cousin	1362	0.08 (0.01, 0.16)	0.06 (-0.01, 0.12)	0.02 (-0.04, 0.08)
Spousal	887	0.03 (-0.04, 0.10)	0.05 (-0.02, 0.11)	-0.02 (-0.08, 0.05)

CRVE, central retinal venule equivalent; CRAE, central retinal arteriole equivalent; AVR, arteriole-to-venule ratio.

TABLE 3. Familial Correlations of Retinal Vessel Equivalents among Subsets of People in the Beaver Dam Eye Study, 1988–1990

Relationship	Number of Pairs	Correlation (95% Confidence Interval)		
		Venule (CRVE)	Arteriole (CRAE)	Ratio (AVR)
All persons without diabetes:				
Sibling	718	0.23 (0.15, 0.32)	0.21 (0.13, 0.30)	0.10 (0.02, 0.19)
Parent-Child	289	0.26 (0.15, 0.37)	0.28 (0.17, 0.39)	0.19 (0.08, 0.30)
Avuncular	456	0.14 (0.03, 0.26)	0.07 (−0.04, 0.18)	0.06 (−0.04, 0.16)
Cousin	1092	0.09 (0.01, 0.16)	0.04 (−0.03, 0.12)	0.02 (−0.05, 0.09)
Spousal	722	0.07 (−0.01, 0.14)	0.04 (−0.03, 0.11)	−0.01 (−0.08, 0.06)
All persons without hypertension:				
Sibling	362	0.27 (0.16, 0.38)	0.17 (0.06, 0.28)	0.21 (0.10, 0.33)
Parent-Child	145	0.26 (0.10, 0.42)	0.29 (0.13, 0.44)	0.05 (−0.12, 0.23)
Avuncular	219	0.19 (0.03, 0.34)	0.14 (−0.01, 0.29)	0.11 (−0.04, 0.26)
Cousin	513	0.14 (0.04, 0.25)	0.07 (−0.03, 0.16)	0.02 (−0.08, 0.12)
Spousal	383	0.05 (−0.05, 0.15)	0.04 (−0.06, 0.14)	−0.04 (−0.14, 0.06)
All persons not currently smoking:				
Sibling	586	0.24 (0.15, 0.33)	0.23 (0.14, 0.32)	0.09 (0.00, 0.18)
Parent-Child	235	0.26 (0.14, 0.39)	0.20 (0.07, 0.32)	0.15 (0.03, 0.28)
Avuncular	372	0.11 (−0.01, 0.23)	0.04 (−0.09, 0.16)	0.10 (−0.01, 0.21)
Cousin	904	0.03 (−0.05, 0.11)	0.04 (−0.04, 0.12)	0.00 (−0.07, 0.07)
Spousal	607	0.02 (−0.06, 0.10)	0.04 (−0.04, 0.12)	−0.07 (−0.15, 0.01)

CRVE, central retinal venule equivalent; CRAE, central retinal arteriole equivalent; AVR, arteriole-to-venule ratio.

and 0.32, respectively. In considering gender specific pairs (data not shown), there were no significant differences between brother–brother, sister–sister, and brother–sister pair correlations. Similarly, there were no differences between the father–daughter, father–son, mother–daughter, and mother–son correlations.

To further evaluate the effect of diabetes, hypertension, and smoking status, we reran the analyses in restricted subgroups of the population (Table 3). In analyses restricted to persons free of diabetes, correlations were very similar to those in the whole population. The same was true when analyses were restricted to persons that were not current smokers. When restricted to persons that were normotensive, the correlations generally increased a little, but the number of pairs dropped substantially, so the confidence limits were quite wide.

Refractive error or other optical phenomena could influence the absolute arteriole and venule calibers. The analyses were rerun adjusting for refraction along with the other covariates and no change to the correlation pattern was found (data not shown). The effect of refractive error was also adjusted for by calculation of the AVR. Since a ratio of two measures typically has higher variance than each individual measure, we considered an alternative approach that does not increase variability. The arteriole caliber was regressed on the venule caliber and the residuals used as a measure of the relative increase/decrease in arteriole caliber. The sibling, parent–child, avuncular, cousin, and spousal correlations from this approach were 0.14, 0.19, 0.06, 0.03, and 0.01, respectively. The heritability was 0.38, which is slightly higher than the heritability estimated using the AVR.

DISCUSSION

The observed correlation patterns were consistent with a genetic influence on retinal vascular diameters. The parent–child and sibling correlations were similar. The avuncular correlations were about half the parent–child correlations, and the cousin correlations were about half again. The spousal correlations were no different from zero. The pattern of correlation most consistent with a genetic component was for the CRVE. Although the correlations were smaller for AVR, the pattern for

this characteristic was also consistent with a genetic component. The CRAE also showed some genetic influence, but may have some additional environmental influence as reflected by a higher sibling correlation than parent–child correlation and similar cousin and avuncular correlations. These same relationships held even in subgroups free of diabetes, among nonsmokers only and among normotensives.

The slightly different pattern of correlations for CRAE may, in part, have been due to blood pressure influences, which would affect arterioles more than venules.^{9,14–16} This did not appear to have affected the AVR correlations, however. We adjusted for blood pressure before analyses, but there still may have been some residual effect. Another possible explanation was that the arteriole measures were more variable than the venule measures. In a small study of the effect of pulse cycle on the arteriole and venule measurements, Knudtson et al.³⁹ found that the larger source of variation on these measures was between graders and between photographs. This was less true, however, for the larger venules. The confidence intervals for the correlations, however, were no wider for the CRAE than for the CRVE in this analysis, so the increased variability of the CRAE did not appear to explain the different correlation pattern.

Refractive error was related to the arteriole and venule equivalent, but not to the AVR.⁴⁰ Since refraction aggregated in families,⁴¹ the higher correlations for CRAE and CRVE compared to the AVR may have reflected different refractive errors. However, adjustment for refractive error did not attenuate the CRAE and CRVE correlations. Another possibility for the lower correlations for AVR may have been the increased variability inherent in ratios. Regression of the arterioles on the venules, rather than considering the AVR, did not increase the correlations to the level of the CRAE and CRVE, however.

Recent epidemiologic data have suggested that generalized retinal arteriolar narrowing may precede the development of hypertension, cardiovascular disease, and stroke. Results in the Atherosclerosis Risk in Communities (ARIC) study,¹³ Cardiovascular Health Study (CHS),¹⁴ Blue Mountains Eye Study,¹⁵ and Beaver Dam Eye Study¹⁶ populations have showed that persons with hypertension have lower AVR, and that, independent of hypertension, AVR was predictive of stroke¹⁸ and

coronary heart disease in women.²⁰ While generalized retinal arteriolar narrowing was thought to represent structural changes resulting from persistent high blood pressure, recent data¹⁷ suggest that generalized retinal arteriolar narrowing may precede hypertension. The data in our present study suggest that, beyond structural changes of vessels due to hypertension, the narrowing of the retinal arterioles may be, in part, genetically determined.

One of the limitations of our analysis was our inability to examine environmental influences and any gene and environment interactions. Observed correlations indicated similarity between relatives. This similarity could be from environmental and/or genetic influences. We adjusted for known measured environmental influences such as blood pressure and smoking, and still had strong sibling correlations. Because of the large number of extended pedigrees, we were also able to examine the pattern of correlations among the types of relatives which suggested stronger genetic, rather than environmental, influences. First-degree relatives, such as siblings and parents and children, have 50% of their genes in common. Second-degree relatives, such as half-siblings, avuncular, and grandparental relationships, share 25% of their genes, and third-degree relatives, such as first cousins, share half that again (12.5%). For a trait determined by a gene, correlations for first-degree relatives would be similar, correlations for second-degree relatives would be half as high, and correlations for third-degree relatives would be half that again. An environmental influence would change these correlation patterns. For example, an environmental influence affecting generations differently would cause sibling and cousin correlations to be higher/lower than expected compared to the parent-child and avuncular relationships. This may have occurred for the CRAE measures, but the CRVE and AVR measures showed correlation patterns consistent with a more genetic model.

In conclusion, evidence was found for a genetic component to retinal vessel caliber. Additional analyses are required to determine mode of inheritance, but multiple genes are probably contributing to retinal vessel caliber. Genes may directly or indirectly (through blood pressure, for example) affect the vessel caliber. Understanding the genetic and possible environmental mechanisms influencing retinal vessel caliber in the eye may provide insights into the role of microvascular disease in the pathogenesis of cardiovascular disease, stroke, and hypertension.

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