Effect of Axial Length on Retinal Vascular Network Geometry

NIALL PATTON, MRCOPHTH, RISHMA MAINI, BSc, TOM MACGILLIVARY, PHD,
TARIQ M. ASLAM, DM, IAN J. DEARY, PHD, AND BALJEAN DHILLON, FRCOPHTH

- PURPOSE: To explore the association between axial length and retinal vascular network geometry (arteriovenous diameter ratio [AVR], arteriolar branching angles, and junctional exponents).
- DESIGN: Prospective, cross-sectional study.
- METHODS: Patients were recruited from a pseudophakic population that had preexisting axial length measurements. Mean arterial blood pressure and previous medical history were recorded. Fundal photographs were taken. Digital image analysis was used to determine the AVR, mean arteriolar bifurcation angle, and junctional exponent for each subject.
- RESULTS: In total, 52 subjects were analyzed. Axial length had no association with AVR (R = −0.01, P = .941), mean angles at arteriolar bifurcations (R = −.134, P = .342), or junctional exponents (R = .003, P = .982). However, increased axial length was associated with a trend for lower measured retinal venular and arteriolar diameters (R = −.28, P = .04 and R = −.23, P = .10, respectively). Junctional exponents correlated with both the AVR (R = .32, P = .019) and vascular bifurcation angles (ω) (R = .317, P = .022).
- CONCLUSIONS: Increased axial length is associated with narrowing of both arteriolar and venular diameters, but not on the AVR, or vessel junctions. Future studies exploring the influence of systemic disease on retinal vascular topography do not need to consider axial length as a potential confounding variable when utilizing measures such as AVR or vessel junctions. Vascular arteriolar junctional exponents may serve as a good measure of overall altered retinal vascular geometry in cardiovascular disease. (Am J Ophthalmol 2005;140:648–653. © 2005 by Elsevier Inc. All rights reserved.)

THE CARDIOVASCULAR SYSTEM IS A HIGHLY branched structure, with the number of blood vessel junctions being in the order of billions. However, contrary to vascular topography being a completely random geometric network, there is evidence to support the concept of an organized geometric structure, to minimize physical properties such as shear stress and work across any vascular network.[1–6] In 1926, Murray calculated that the most efficient circulation across a vascular network can be achieved if blood flow is proportional to the cubed power of the vessel's radius (known eponymously as Murray's law),[2] assuming the blood acted as a Newtonian fluid, that blood viscosity was constant throughout the vascular network, and that metabolism of the blood and vessel tissue also remains constant within the vascular system. Thus, $D_0^X = D_1^X + D_2^X$, where (D_0) is the diameter of the parent vessel, and (D_1 and D_2) are the diameters of the daughter vessels. Theoretical values for the value of ^{X} (junctional exponent) approximate to the value of 3 in healthy vascular networks, and deviations from this optimal value reflect a less optimized circulatory network. In addition, the angle subtended between two daughter vessels at a vascular junction has also been found to be associated with an optimal value, approximately 75 degrees.[6] Thus, these parameters serve as measures of optimality of circulatory geometry and can be used to obtain information on the effect of systemic cardiovascular disease on the retinal circulation.

Other geometric features of the retinal circulation that have been studied include the measurement of retinal vessel widths.[7,8] However, any measurement taken from a fundal image is subject to the magnification effect of the eye,[9] which will depend on, among other things, the refractive error of the eye. Therefore, the use of dimensionless entities such as the arteriovenous ratio (AVR)[7] has been calculated as generalized representations of vessel widths across the retinal vascular circulation.

Whereas a large number of studies have explored the relationship between retinal vascular network geometry

and systemic cardiovascular disease,[7,8,10–14] few studies have explored the influence of ocular factors on retinal vascular geometry. There is some evidence to support the concept of axial length being related to retinal vascular topographic features such as length:diameter ratios of retinal arterioles[15] and that axial length may have an influence on retinal blood flow.[16] Recent epidemiologic studies have found no relationship between refractive error and AVR,[17,18] although a decreased arteriolar and venular diameter was reported to be associated with increased myopia.[18]

We performed a prospective study, exploring any possible relationship between axial length and retinal topographic features of AVR, the angles between daughter vessels at dichotomous vascular junctions, and junctional exponents. We chose axial length, because this may more accurately reflect any influence myopia may have on retinal topography, as opposed to the subject's refraction, which will also be influenced by corneal factors. Because our population was pseudophakic (with an aimed emmetropic refraction), magnification effect due to refractive error might be expected to play less of a role.
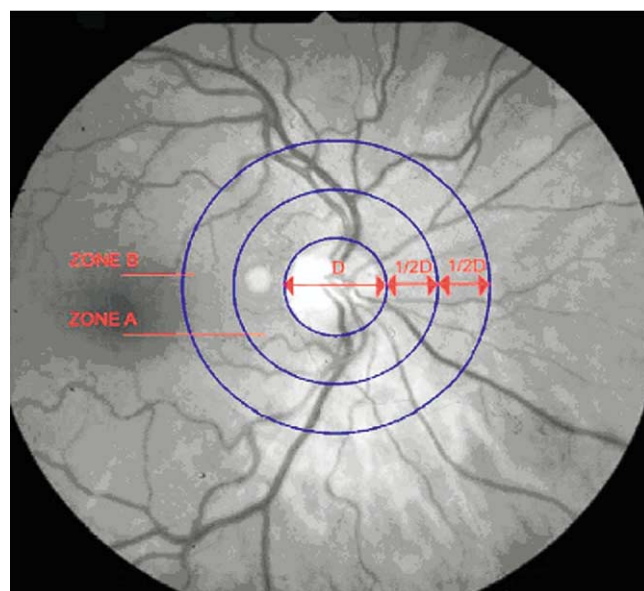

FIGURE 1. A typical fundus image that has been converted from a color to a greyscale image and undergone contrast enhancement. An Atherosclerosis Risk in Communities grid demonstrating the region between 0.5 and 1 disk diameters from the edge of the optic disk has been superimposed on the image, and the widths of all arterioles and venules passing through this region (zone B) will be measured to obtain the arteriovenous ratio.

METHODS

THE STUDY WAS APPROVED BY THE LOCAL INSTITUTIONAL review board, and full written informed consent was obtained from all participants. Consecutive pseudophakic patients attending general outpatient clinics were recruited. Factors that were recorded include male or female sex, age, smoking status (never, former, current), use of vasoactive medications (see explanation that follows), and presence of cardiovascular history (previous stroke, ischemic heart disease, peripheral vascular disease). In all subjects before retinal photography, blood pressure was recorded with use of an automated sphygmomanometer. Axial length had been measured preoperatively in all patients with A-scan biometry. Exclusion criteria included a past medical history of a retinal vascular event, presence of diabetic retinopathy, hypertensive retinopathy, and any retinopathy of unknown etiology. Also excluded were any patients in whom the accuracy of the precataract axial length measurement was questionable, any patients unable to give full informed consent, and any patients in whom we were unable to obtain an analyzable fundal image.

Medications were classified as vasodilators (calcium channel antagonists, nitrates, potassium channel antagonists, β-adrenoreceptor agonists, angiotensin II antagonists, angiotensin-converting enzyme inhibitors, α-adrenoreceptor antagonists) or vasoconstrictors (β-adrenoreceptor antagonists). If a subject was taking vasoconstrictor and vasodilator medication, the net effect was taken to be vasodilation, because there are few β-adrenoreceptors in the retinal vasculature.

All participants had their pupils pharmacologically dilated with tropicamide 1%; 35 mm color photographs were taken with a mydriatic retinal camera (Kowa Pro 1 professional fundus camera, Japan) with a 50-degree field of view centered on the optic disk. After film processing, the 35 mm photographs were then digitally scanned at 3200 dpi with an Epson 3200 Perfect Scanner and stored in Tagged Image File Format (TIFF).

Digital image analysis was performed by means of a custom-written application package within the MATLAB version 6.0 (The Mathworks Inc, Natick, Massachusetts) environment. Measurements were performed on a Sun Microsystems, Inc (Palo Alto, California) computer workstation with a high-resolution 19-inch monitor by one trained observer. The color RGB image was converted to a grayscale image, and image contrast was enhanced by using contrast limited adaptive histogram equalization (CLAHE).

In accordance with the Atherosclerosis Risk in Communities (ARIC) study, a concentric circular grid centered on the optic disk was overlaid to define the measurement zone. All vessels passing completely through zone B (.5 to 1.0 disk diameter from the disk margin) were measured (Figure 1). Vessel edges were identified by using the Canny edge detection program. The Canny edge algorithm[19] smoothes the image by using a Gaussian filter to eliminate noise. It then finds the edge strength by taking the gradient of the image to highlight regions with high spatial deriv-

atives. The observer chose the segment of the vessel to be measured, choosing a segment of uniform width (avoiding segments of focal narrowing or distention), and this region of interest was magnified, enabling the vessel edges to be selected by means of a mouse-driven cursor. Each vessel was identified as an arteriole or a venule with use of the original color slide for reference.

Parr and Spears[20] developed a quantitative technique of calculating the width of the central retinal artery that is independent of the number and pattern of branching of the larger arteries of the retina and can therefore be used to compare the arterial caliber of different eyes. They expressed this value as the central retinal artery equivalent (CRAE). Hubbard and associates[7] developed a similar formula for central retinal vein equivalent (CRVE). The ARIC study devised a simplified procedure for combining measurements to yield central equivalents, and the same technique was applied here. Finally, the summary indices of the arteriolar and venular diameters were expressed as an AVR. To convert the dimensions measured on the image (pixels) into actual measurements (micrometers), we based the average optic disk diameter as 1850 μm (as in the ARIC study),[7] and therefore the mean optic disk diameter in this series of images was converted by multiplying the value in pixels by 12.67, to obtain a measure in micrometers. (The average disk diameter was 146 pixels.)

For each subject, the four most proximal arteriolar bifurcations (representing the first bifurcation of the superonasal, superotemporal, inferonasal, and inferotemporal arterioles) from the optic disk margin were evaluated. The mean angle and junctional exponent for each subject was calculated from these data. On occasion, we were unable to obtain a value for a major arteriolar junction, either because the bifurcation was being masked by an overlying venule, or because it occurred beyond the 50-degree fundal field. In this case, the three most proximal arteriolar bifurcations were recorded, and the mean angle and junctional exponent were calculated. The bifurcation angle (ω) was measured as the angle between the two daughter arterioles.[21] The widths of the parent and daughter vessels were calculated by drawing a line normal to the vessel edge and deriving the mean value of five parallel image profile cross-sections, each cross-section being two pixels away from its neighbor. Each cross-section was matched to a double-Gaussian[22] profile with use of Matlab image-processing toolbox and curve-fitting toolbox. The width of the vessel was taken at half the height of the fitted curve.[23] The measurement was rejected and repeated if the standard deviation of the five calculated widths was greater than 10% of the mean width measured, or if any of the five fitted curves were on inspection deemed to be inaccurate. The junctional exponent was then calculated from the widths of the parent and daughter vessels by using an iterative computer-driven process.

Pearson's product-moment correlation coefficient was used to assess any relationship between axial length and

the values of the AVR, bifurcation angle, or junctional exponents. In addition, stepwise multiple linear regression models were constructed with AVR, bifurcation angles, and junctional exponents as dependent variables. Statistical significance was at the $P < .05$ level. The intraobserver repeatability for the digital imaging measurements, expressed as within-subject standard deviation,[24] was 1.41 pixels for diameter measurements (mean diameter 22.3 pixels) and was 2.13 degrees for angle of bifurcations (mean angle 78.4 degrees). For the AVR measurements, the "limits of agreement" technique was used. Mean difference was 0.007, with 95% of measurements falling within the range +0.125 to −0.112. Graphic examination of the Bland-Altman plot showed no systematic bias with change in AVR.

RESULTS

IN TOTAL, 57 PATIENTS WERE RECRUITED AND UNDERWENT retinal photography. Five patients were excluded on account of poor fundal image quality, leaving 52 patients for analysis. Of the 52 patients, 22 were men (mean 70 years; range 43 to 84 years) and 30 were women (mean 73 years; range 34 to 94 years). Length of time from cataract surgery varied from 6 weeks to 12 months (median 3 months). Mean arterial blood pressure was 100 mm Hg (SD ± 12.5 mm Hg). Of the 52 patients, 22 had no history and 30 had a history of cardiovascular disease. Three patients were classified as taking vasoconstrictors and 17 as taking vasodilators; 32 were taking no vasoactive medication.

The mean axial length was 23.4 mm (SD ± 1.3 mm, range 21.2 to 28.3 mm). For this population, the mean AVR was 0.86 (SD 0.10, range 0.61 to 1.08). The mean arteriolar bifurcation (ω) was 77.3 degrees (standard deviation ± 9.5 degrees, range 52 to 98 degrees). Mean junctional exponent was 2.19 (SD ± 0.4, range 1.25 to 3.20).

Pearson's product-moment correlation showed no significant association between axial length and AVR (R = −.01, P = .941), bifurcation angle (R = −.134, P = .342), or junctional exponent (R = .003, P = .982). A multiple linear stepwise regression model was constructed, with AVR as the dependent variable, and junctional exponent, age, history of cardiovascular disease, smoking status, vasoactive medications, and mean arterial blood pressure (MABP) as predicting variables. The only association identified was that junctional exponents correlated strongly with the AVR (R = .324, P = .019) (Figure 2).

Mean arterial blood pressure was also associated with junctional exponents (R = .337, P = .015) and AVR (R = −.26, P = .06). Junctional exponents also correlated with bifurcation angles (ω) (R = .317, P = .022).

Both the CRVE and CRAE were associated with a negative trend with respect to axial length, although the CRAE
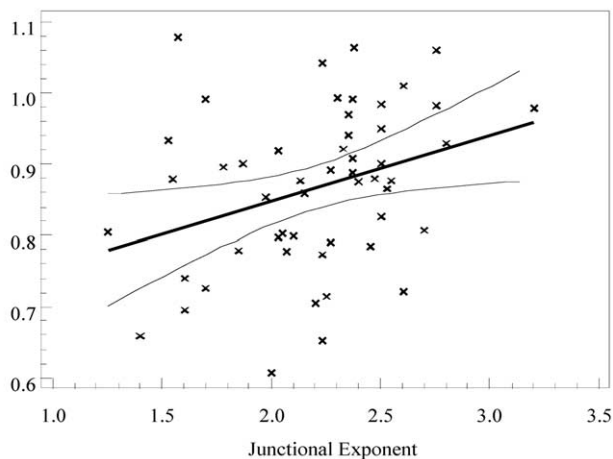
FIGURE 2. X-Y scatterplot diagram demonstrating the linear relationship between the arteriovenous ratio and mean junctional exponent for each individual (n = 52). Solid line = line of regression, (R = .324, P = .019). Dashed lines = 95% confidence intervals.
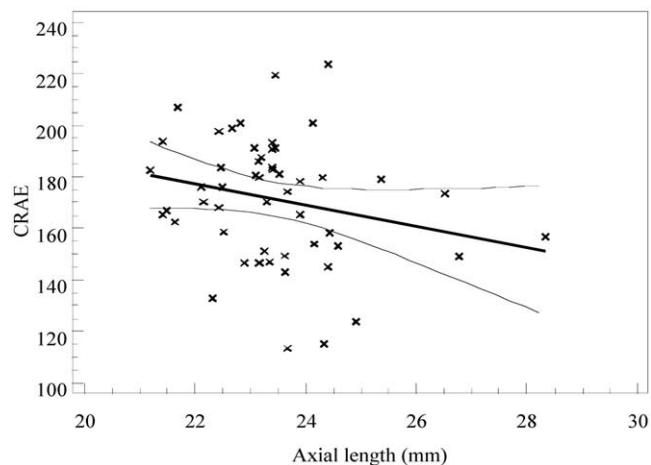

FIGURE 4. X-Y scatterplot demonstrating the inverse linear relationship between the central retinal arterial equivalent (CRAE) and the axial length in millimeters for each individual (n = 52). Solid line = line of regression (R = −.23, P = .10). Dashed lines = 95% confidence intervals.
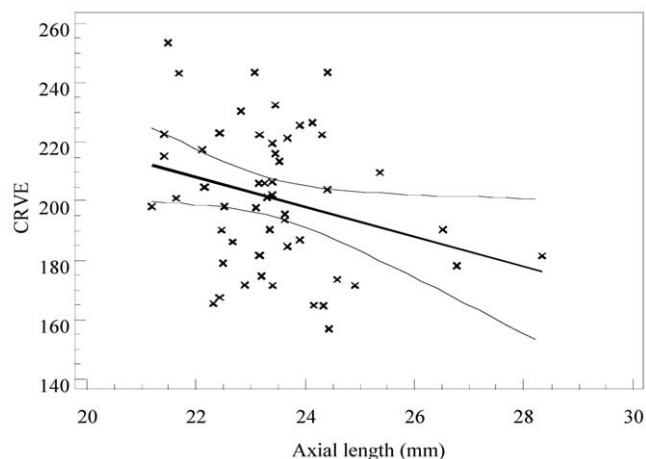

FIGURE 3. X-Y scatterplot diagram demonstrating the inverse linear relationship between the central retinal vein equivalent (CRVE) and the axial length in millimeters for each individual (n = 52). Solid line = line of regression (R = −.28, P = .04). Dashed lines = 95% confidence intervals.

did not reach statistical significance (R = −.28, P = .04 and R = −.23, P = .10, respectively) (Figures 3 and 4).

DISCUSSION

A LARGE NUMBER OF STUDIES HAVE EXPLOITED DIGITAL retinal image analysis to explore the relationship between retinal vascular topography and systemic disease.[7,8,10–14] However, few studies have explored the possible ocular factors that may have a bearing on retinal vascular topography.[17,18] Some evidence supports the concept of axial length being related to retinal topographic features and retinal blood flow.[16] Central retinal arterial blood flow is reduced in myopic eyes,[25,26] and retinal blood flow has been found to be inversely proportional to axial length in a glaucomatous population,[27] although no relationship was found in normal and ocular hypertensive groups. There is a possibility that this influence of axial length on retinal blood flow is partly mediated through changes in the retinal vascular network geometry.

Our primary objective in this study was to examine the association of axial length with dimensionless parameters of retinal vascular network geometry—the AVR, angles at vessel bifurcations, and junctional exponents. Our finding of no association with either of these parameters suggests that for future studies exploring the effect of systemic cardiovascular risk factors on the retinal vascular network, axial length does not need to be considered as a confounding variable. Our findings agree with a recently published epidemiologic study that found no influence of refractive error on the relationship between retinal vascular diameters and hypertension or on absolute retinal vessel diameters.[17] In our study, we used axial length, as we hypothesized that the actual length of the eye may have a greater bearing on retinal vascular topography than refraction, because refraction will also be influenced by corneal factors.

Interestingly, both the CRVE and CRAE in our study were associated with a negative trend with respect to increased axial length. The Blue Mountains Eye Study[17] also found that correcting for refractive error with the Bengtsson method[28] did appear to increase the statistical power to detect associations with retinal vessel diameters, and the investigators recommended for future studies that refraction might be taken into consideration. In addition, they highlighted the need for future studies with axial

length data to explore more precisely its impact on retinal vascular diameters and their association with systemic cardiovascular disease. The Beaver Dam Eye Study[16] also found that myopic refraction was associated with smaller retinal vessel diameters, but there were no data on axial length in this study, and the authors speculate as to whether this is purely a magnification effect, or whether it represents a biologic or pathologic process in eyes with different refractions. We believe our study supports evidence that narrowed retinal vessel diameters in association with increased axial length may represent an actual biologic process, rather than merely a magnification effect. This is the first study to find an association between axial length and retinal vessel diameters. A limitation of our study is that there is no information regarding the actual refraction of the subjects. However, all patients were pseudophakic with an aimed refraction of emmetropia, and hence the refraction might be suspected to have less of an influence in this population. It also explains why parameters such as the AVR are seemingly unaffected by increased axial length, as both venules and arterioles may be similarly attenuated.

Narrowed retinal vessels may explain the altered retinal blood flow seen in myopia. Quigley and Cohen[15] developed a measure of retinal topography that is derived from Poiseuille's law, Ohm's law, and Murray's law. This "pressure attenuation index" (PAI) reduces to the length:diameter ratio of retinal arterioles and was directly related to axial length (R = 0.65). Therefore, the greater arteriolar length associated with myopia may lead to low end-arterial pressure and thus be the mechanism of protection from diabetic retinopathy due to decreased retinal vascular perfusion.[29] Our findings suggest that in addition to greater arteriolar length, narrowed arterioles may also contribute to this pressure attenuation. Others consider a possible genetic influence on myopia and risk of diabetic retinopathy.[30]

There was an association between MABP and both junctional exponents and AVR. The junctional exponents also correlated with the AVR and vascular bifurcation angles, providing further evidence that geometric retinal vascular junctions reflect a generalized altered cardiovascular systemic state. Indeed, junctional exponents have advantages over the AVR as a potential measure of generalized retinal vascular geometry, because fewer measurements are taken and therefore it is less time consuming to calculate than the AVR. Whereas junctional exponents have not been as utilized in large epidemiologic studies as much as the AVR, they have been found to be associated with aging,[31] vasoconstriction,[32] and peripheral vascular disease[33] and offer a potential alternative measure of retinal vascular geometry.

In conclusion, this is the first study to explore the relationship between axial length and retinal vascular network geometry. Whereas axial length may be associated with narrowed retinal arterioles and venules, overall di-

mensionless measures of vascular network topography are not affected and hence axial length does not need to be taken into consideration when assessing systemic factors likely to affect retinal vascular topography. In addition, junctional exponents reflect both the AVR and angles at retinal vascular junctions and thus may serve as a good measure of overall altered retinal vascular geometry related to systemic cardiovascular disease.

REFERENCES

1. Murray C. The physiological principle of minimum work 1. The vascular system and the cost of blood volume. Proc Natl Acad Sci U S A 1926;12:207–214.
2. Murray C. The physiological principle of minimum work applied to the angle of branching arteries. J Gen Physiol 1926;9:835–841.
3. Zamir M. Optimality principles in arterial branching. J Theor Biol 1976;62:227–251.
4. Zamir M, Medeiros J, Cunningham TK. Arterial bifurcations in the human retina. J Gen Physiol 1979;74:537–548.
5. Zamir M, Medeiros J. Arterial branching in monkey and man. J Gen Physiol 1982;77:353–360.
6. Sherman T. On connecting large vessels to small: the meaning of Murray's law. J Gen Physiol 1981;78:431–453.
7. Hubbard LD, Brothers RJ, King WN, et al. Methods for evaluation of retinal microvascular abnormalities associated with hypertension/sclerosis in the Atherosclerosis Risk in Communities Study. Ophthalmology 1999;106:2269–2280.
8. Sherry LM, Wang JJ, Rochtchina E, et al. Reliability of computer-assisted retinal vessel measurement in a population. Clin Exp Ophthalmol 2002;30:179–182.
9. Arnold JV, Gates JWC, Taylor KM. Possible errors in the measurement of retinal lesions. Invest Ophthalmol Vis Sci 1993;34:2576–2580.
10. Chapman N, Mohamudally A, Cerutti A, et al. Retinal vascular network architecture in low birth weight males. J Hypertens 1997;15:1449–1453.
11. Wong TY, Klein R, Klein BEK, Tielsch JM, Hubbard LD, Nieto FJ. Retinal microvascular abnormalities and their relationship with hypertension, cardiovascular diseases and mortality. Surv Ophthalmol 2001;46:59–80.
12. Wong TY, Klein R, Sharrett AR, et al. Retinal arteriolar diameter and risk for hypertension. Ann Intern Med 2004; 17:248–255.
13. Wong TY, Klein R, Couper DJ, et al. Retinal microvascular abnormalities and incident stroke: the Atherosclerosis Risk in Communities Study. Lancet 2001;358:1134–1140.
14. Smith W, Wang J, Wong TY, et al. Retinal arteriolar narrowing is associated with 5-year incident severe hypertension: the Blue Mountains Eye Study. Hypertension 2004; 44:442–447.
15. Quigley M, Cohen S. A new pressure attenuation index to evaluate retinal circulation. Arch Ophthalmol 1999;117:84–89.
16. Ravalico G, Pastori G, Croce M, Toffoli G. Pulsatile ocular blood flow variations with axial length and refractive error. Ophthalmologica 1997;211:271–273.

17. Wong TY, Wang J, Rochtchina E, Klein R, Mitchell P. Does refractive error influence the association of blood pressure and retinal vessel diameters? The Blue Mountains Eye Study. Am J Ophthalmol 2004;137:1050–1055.

18. Wong TY, Knudtson M, Klein R, Klein B, Meuer S, Hubbard L. Computer-assisted measurements of retinal vessel diameters in the Beaver Dam Eye Study—methodology, correlation between eyes and effect of refractive errors. Ophthalmology 2004;111:1183–1190.

19. Canny J. A computational approach to edge detection. IEEE Transactions on pattern analysis and machine intelligence 1986;8:769–798.

20. Parr JC, Spears GF. General calibre of the retinal arteries expressed as the equivalent width of the central retinal artery. Am J Ophthalmol 1974;77:472–477.

21. Chapman N, Haimes G, Stanton A, Thom S, Hughes A. Acute effects of oxygen and carbon dioxide on retinal vascular network geometry in hypertensive and normotensive subjects. Clin Sci 2000;99:483–488.

22. Gao XW, Bharath A, Stanton A, Hughes A, Chapman N, Thom S. Quantification and characterization of arteries in retinal images. Comput Methods Programs Biomed 2000;63:133–134.

23. Newsom R, Sullivan P, Rassam S, Jagoe R, Kohner E. Retinal vessel measurement: comparison between observer and computer driven methods. Graefes Arch Clin Exp Ophthalmol 1992;230:221–225.

24. Bland J, Altman D. Measuring agreement in method comparison studies. Stat Methods Med Res 1999;8:135–160.

25. Dimitrova G, Tamaki Y, Kato S, Nagahara M. Retrobulbar circulation in myopic patients with or without myopic choroidal neovascularisation. Br J Ophthalmol 2002;86:771–773.

26. Shimada N, Ohno-Matsui K, Harino S, et al. Reduction of retinal blood flow in high myopia. Graefes Arch Clin Exp Ophthalmol 2004;242:282–288.

27. Nemeth J, Michelson G, Harazny J. Retinal microcirculation correlates with ocular wall thickness, axial eye length and refraction in glaucoma patients. J Glaucoma 2001;10:390–395.

28. Bengtsson B. The variation and covariation of cup and disc diameters. Acta Ophthalmol (Copenh) 1976;54:804–818.

29. Pierro L, Brancato R, Robino X, Lattanzio R, Jansen A, Calori G. Axial length in patients with diabetes. Retina 1999;19:401–404.

30. Baker R, Rand L, Krolewski A, Maki T, Warram J, Aiello L. Influence of HLA-DR phenotype and myopia on the risk of nonproliferative and proliferative diabetic retinopathy. Am J Ophthalmol 1986;102:693–700.

31. Stanton AV, Wasan B, Cerutti A, et al. Vascular network changes in the retina with age and hypertension. J Hypertens 1995;13:1724–1728.

32. Griffith T, Edwards D, Randall M. Blood flow and optimal vascular topography: role of the endothelium. Basic Res Cardiol 1991;86(suppl 2):89–96.

33. Chapman N, Dell'omo G, Sartini MS, et al. Peripheral vascular disease is associated with abnormal arteriolar diameter relationships at bifurcations in the human retina. Clin Sci 2002;103:111–116.

Biosketch

Niall Patton, MD, is an ophthalmologist at Princess Alexandra Eye Pavilion, Edinburgh, United Kingdom. Currently, he is completing a one year fellowship in vitreoretinal surgery at Sir Charles Gairdner Hospital and the Lions Eye Institute, Perth, Western Australia. Research interest includes image processing and analysis in ophthalmology, particularly retinal vascular image analysis.

The authors of this manuscript are a group of researchers currently undertaking studies exploring the relationship between ocular and systemic factors on retinal vascular network geometry, in particular retinal vascular network geometry and cognitive function in an elderly population. This research is being carried out in Edinburgh, United Kingdom, and is being led by Professors Baljean Dhillon and Ian J. Deary.