Heritable Features of the Optic Disc: A Novel Twin Method for Determining Genetic Significance

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Purpose. Numerous genetic diseases and environmental stimuli affect optic nerve morphology. The purpose of this study was to identify the principal heritable components of visible optic nerve head structures in a population-based sample of twins.

Methods. Fifteen optic nerve specialists viewed stereoscopic optic nerve head photographs (Stereo Viewer-II; Pentax Corp., Tokyo, Japan) from 50 randomly selected monozygotic or dizygotic twin pairs. Before viewing, each expert was questioned about which optic nerve head traits they believed were inherited. After viewing a standardized teaching set, the experts indicated which twin pairs they thought were monozygotic. Participants were then questioned about how their decisions were reached. A rank-ordered Rasch analysis was used to determine the relative weighting and value applied to specific optic nerve head traits.

Results. The proportion of twin pairs for which zygosity was correctly identified ranged from 74% to 90% (median, 82%) across the panel. Experts who correctly identified the zygosity in more than 85% of cases placed most weighting on shape and size of the optic disc and cup, whereas experts with the lowest scores placed greater weighting on the optic nerve head vasculature in reaching their decisions.

Conclusions. In determining the genetic components of the optic nerve head, the results of this study suggest that the shape and size of the optic disc and cup are more heritable and should receive a greater priority for quantification than should vascular features. (*Invest Ophthalmol Vis Sci.* 2007;48: 2469–2475) DOI:10.1167/iovs.06-1470

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Understanding the principal factors that contribute to variation in human traits is important, because genetic or environmental determinants of these traits may also be related to disease susceptibility. Quantitative traits are inherently more powerful for disease loci identification than attempting to map dichotomous (present/absent) diseases such as glaucoma, given their relative rarity in the population.[1]

The optic nerve head (ONH), which is bounded by Elschnig's ring as demarcated by the termination of Bruch's membrane, is composed of numerous quantifiable anatomic structures.[2] Ganglion cell axons converge at the ONH, to exit the eye in a crude retinotopographic pattern.[3] Similarly, retinal arteries and veins either enter or leave the eye, respectively, through this scleral foramen. Various diseases with a genetic basis manifest clinically in the form of altered architecture of both the intra- and parapapillary regions of the ONH.[4] It would be helpful to understand which ONH characteristics should be prioritized for quantitative trait loci (QTL) analysis.

In the prioritization of ONH traits for QTL investigation, it should be remembered that different disease processes may result in the same phenotypic appearance, but also that pleiotropy may occur, in that a single disease may cause a variable ONH phenotype. For example, in addition to the classic glaucomatous optic cup excavation (which can be focal or diffuse) one may see retinal artery narrowing and expansion of the zone of β-parapapillary atrophy in primary open-angle glau-

coma.[2,5] In addition, other genetic diseases cause other abnormalities of the ONH, such as the presence of telangiectatic vessels in some patients with Leber hereditary optic neuropathy.[6] Nevertheless, the genes underlying the many diseases that affect the ONH may be identified through investigating its chief heritable components.

Common variation (or nucleotide polymorphisms) in a gene may influence its expression, which in part explains the normal variance of trait. However, other variants may completely disrupt a gene's function, thereby precipitating a diseased state. The work by Zhu et al.[7] serves as a case in point for using such a model to identify genes involved in complex diseases. They found that a large proportion (74%) of the genetic liability for normal variation in eye color is due to a QTL in the *OCA2* gene, a gene previously implicated in causing oculocutaneous albinism.[8] Mutations that completely disrupt the function of both copies of the *OCA2* gene result in the disease phenotype of oculocutaneous albinism.[8] Recently, fine mapping of the *OCA2* gene in discordant twins has identified three common polymorphisms in intron 1 that account for this large genetic effect that determines normal eye color in the general population.[9]

The purpose of this study was to determine which features of the ONH are primarily genetically determined. ONH photographs from randomly selected twin pairs were viewed by practitioners who have a subspecialist interest in the optic nerve. These experts, who were masked to zygosity, attempted to identify which pairs they thought were monozygotic (MZ) and which they thought were dizygotic (DZ). The underlying premise was that the ONH traits that are most highly heritable would be those on which the experts who were the most proficient at correctly identifying twin-pair zygosity based their decisions. In a second experiment, a random series of an additional set of MZ twins were selected, and the right ONH photograph was displayed, with the expert then randomly viewing either the same person's left ONH, flipped horizontally to appear as a right ONH, or the right ONH from the MZ pair. Each expert was asked to nominate which of the latter two most resembled the ONH first viewed. We reasoned that major epigenetic factors would account for variation in ONH morphology should each expert be able to nominate the ONH photographs from the same person consistently. Conversely, mirroring or laterality would be important in ONH embryogenesis if the experts were consistently nominating the right ONH photographs from the MZ pair as being most similar. Overall, the results of this study allow for the prioritization of quantifiable ONH traits for further genetic investigation.

Materials and Methods

Subjects Recruitment and Study Protocol

Twin pairs were identified as part of the Twins Eye Study in Tasmania (TEST) and were recruited from the general population through local media campaigns as well as through a national registry. The Australian twin registry includes more than 30,000 sets of twins. Invitations were then were sent directly to all registered Tasmanian twins (>1000 eligible sets). The relevant ethics committees of the University of Tasmania as well as the Royal Victorian Eye and Ear Hospital approved the study, and the protocol adhered to the tenets of the Declaration of Helsinki. Each subject or his or her respective legal guardian provided written informed consent before participation.

All recruited twins underwent a comprehensive clinical examination that included anterior segment examination, intraocular pressure measurement, corneal pachymetry, refraction, and a mydriatic optic disc assessment. Simultaneous stereoscopic optic disc photographs were obtained with a fundus camera (3-Dx/F; Nidek, Gamagori, Japan) on 35-mm slide film (Ektachrome; Eastman Kodak, Melbourne, Australia). For all twin pairs, zygosity was determined by DNA analysis with the following polymorphic microsatellite markers: *D2S2211* (7 alleles); *D3S1267* (13 alleles); *D6S257* (11 alleles); *D8S284* (8 alleles); *D11S4151* (6 alleles); *D12S345* (10 alleles); *D14S283* (9 alleles), and *D17S1852* (12 alleles). According to the models developed by Nyholt, our genotyping protocol would falsely classify a DZ pair as MZ in 1 of 4907 cases.[10]

Seventy-seven twin pairs were selected at random from the complete TEST set ($n > 400$). The mean ± SD age of the selected twins was 30.6 ± 11.8 years (range, 7–63 years). Color 35-mm slides of each subject's ONH were viewed (Stereo Viewer-II (Pentax Imaging Company, Golden, CO). All identifying information was removed from each stereoscopic slide before grading.

Experiment Design

After an initial pilot (viewed by RLC, PLK, CJH, and SSH), 15 optic disc experts (WLA, SLB, WMB, JEC, JHF, PJF, DG-H, CMG, JBJ, NRM, WHM, NJN, HAQ, JRS, GLS), masked to zygosity, viewed the selected slides. Before viewing the slides, these experts were questioned in an unstructured manner about ONH traits that they believed were inherited. Then, after viewing a standardized teaching set of slides from 5 pairs of MZ and 5 pairs of DZ twins, the experts were asked to indicate, in a forced-choice manner, the zygosity of 50 twin pairs. On completion of the full set, experts were questioned qualitatively about how their decisions were reached and then were asked to weight (between 0 and 10) quantitatively the relative importance assigned to specific ONH traits. These specific ONH traits included CDR, optic disc size, optic cup size, optic cup depth, optic disc shape, and optic cup shape, along with overall neuroretinal rim appearance, retinal vessel diameters, location of the vascular trunk, vascular pattern within the optic disc margin, vascular pattern beyond the optic disc margin, the presence of cilioretinal vessels, and the presence of parapapillary atrophy.

In the second experiment, each expert was shown ONH photographs from 17 sets of MZ twin pairs. The experts then viewed a right ONH photograph from one of the MZ twin pairs and attempted to determine whether the same individual's left ONH, flipped horizontally

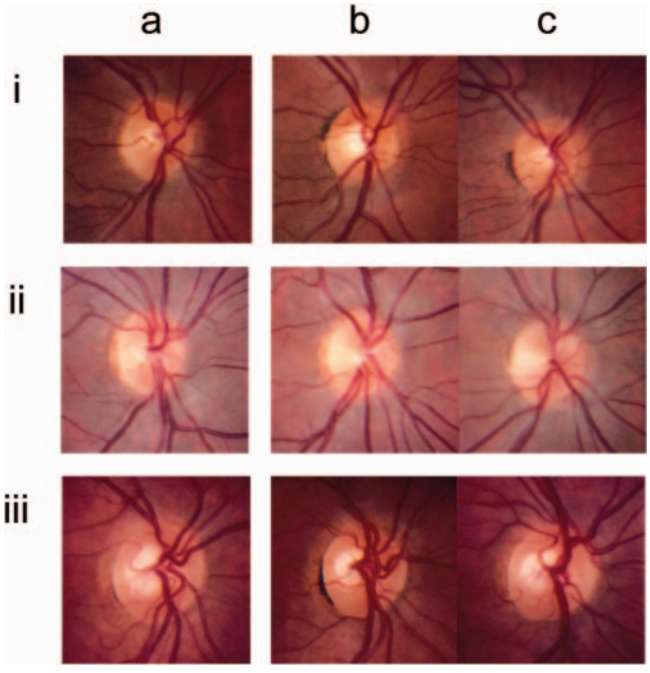

Figure 1. ONH photographs of MZ twin pairs for which each expert was asked, after viewing the right ONH from one of the MZ twin pairs (**a**), whether the same individual's left ONH, flipped horizontally to appear as a right ONH, or the right ONH from the MZ twin, most resembled the first. In examples (**i**) and (**iii**), ONH photographs (**a**) and (**c**) were from the same individual, whereas in (**ii**), photographs (**a**) and (**b**) are from the same individual.

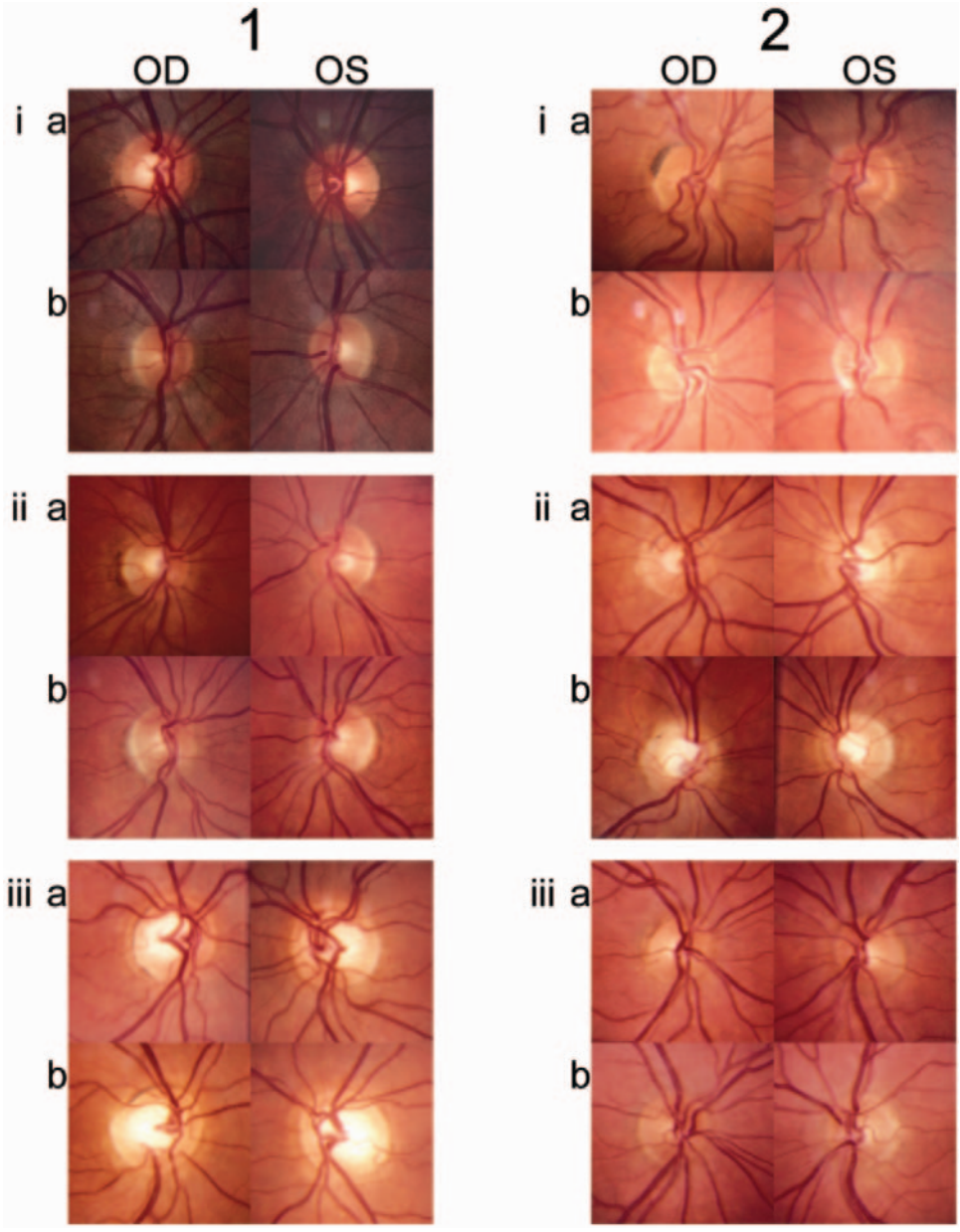

FIGURE 2. ONH photographs of twin pairs. *Column 1*: examples of twin pairs in whom the zygosity was identified correctly by each expert; *column 2*: examples of twin pairs in whom zygosity was most frequently incorrectly nominated. In both columns, twin pair (**i**) was DZ, whereas twin pairs (**ii**) and (**iii**) were MZ.

to appear as a right ONH, or the right ONH from the fellow MZ twin most resembled the first photograph (Fig. 1).

Data Analysis

As a measure of reproducibility and to test for fatigue, the proportion of correctly identified twin pairs in the first set of 25 was compared with that in the last set of 25 by the Fischer exact test (SPSS 12.0.1; SPSS Inc, Chicago, IL).

The responses to the postexperiment structured questionnaire were analyzed by using a Rasch approach (WinSteps 3.61.1 program; WinSteps, Chicago, IL).[11] Rasch analysis allowed the usefulness of specific ONH traits, as weighted by different graders, to be measured on a common logit scale, thereby allowing direct comparison. The Rasch model does not assume values for response categories (e.g., 0, 1, 2. . .) rather it assumes that all categories are on the same underlying latent variable.[12] Categories that were disordered or underutilized were collapsed into adjacent categories. Category probability curves were reviewed to ensure goodness of fit in the probability of observing the relative weighting of each collapsed category at each point on the latent ONH variable.[13] A rank-ordered analysis was used, whereby each grader's ability was rated by the percentage of twin pairs for whom they had nominated the correct zygosity.[14] This percentage then was empirically adjusted from proportions to logits to allow sensible fit statistics. The *t*-standardized, mean-square statistics that are used to compare the predicted responses with the observed were reviewed, to monitor the compatibility of the data with the Rasch model. Outlier-sensitive fit (outfit) mean square is the conventional sum of squared standardized residuals and is sensitive to occasional responses that differ from the expected response, whereas for the information-weighted fit (infit) mean square, each squared standardized residual value is first weighted by its variance and then summed, so as to tolerate extreme responses.[13] Values predictive of measurement are deemed to fall generally between 0.5 and 1.5.[13] The first section of the experiment can be performed online at www.twinseyestudy.com.

RESULTS

The proportions of correctly nominated twin-pair zygosities ranged from 74% to 90% (median 82%) across the 15 graders.

TABLE 1. Relative Score and Responses from Each Ophthalmic Expert

Expert	Correct Zygosity Nominations (%)	MZ Twins Nominated as DZ (*n*)	DZ Twins Nominated as MZ (*n*)	Pre-experiment Response	Postexperiment Response	Correct MZ Matching (%)
A	90	3	2	Size and shape of disc and cup; tilting of disc; depth of cup; slope/profile of neuroretinal rim; vessel pattern; myopic crescent may be useful	Shape and depth of cup; shape and tilting of disc; angulation of vessels through the lamina; vessel pattern not useful; cilioretinal vessels not useful	47.1
B	90	3	2	Size of disc; size and shape of cup; vessel arrangement; scleral crescent may be useful; clarity of neuroretinal rim; neuroretinal rim shape	Orientation of disc; size of disc; size of cup; shape and site of prominence of neuroretinal rim; vessel pattern not useful	70.6
C	86	4	3	Size and shape of disc; vessel trunk course; depth and shape of cup; parapapillary atrophy may be useful	Shape and size of disc; depth, shape and size of cup; location of highest region of nerve fiber layer; parapapillary atrophy	76.5
D	86	5	2	Size of disc; tilting and shape of disc; depth of cup/nerve head; CDR; vasculature features not likely to be useful	Shape, size and orientation of disc; elevation of neuroretinal rim; trunk position in nerve	58.8
E	86	5	2	CDR; location and branching of vessels; titling and shape of disc; color of optic nerve (relative temporal pallor)	Shape of disc; depth of cup; branching pattern of vessels not useful	70.6
F	84	5	3	Size and shape of disc; size of cup; cilioretinal vessels may be useful; arrangement and direction of vessels may be useful	Size of disc; orientation of disc; shape and depth of the cup; orientation of the vessel trunk	58.8
G	84	4	4	Size of disc; size and shape of cup; tilting of disc; parapapillary atrophy; vascular branching pattern likely not to be useful	Size and shape of disc; pattern of vessels outside the disc margin; nerve fiber layer volume not useful	64.7
H	82	6	3	Size and shape of cup; size and shape of disc; temporal vessel branching pattern, parapapillary pigmentation; depth of trunk branching	Shape and size of cup, shape and size of disc; presence of cilioretinal vessels; pattern of vasculature	70.6
I	82	4	5	Vascular pattern; position of vascular branching; size of disc; site of maximum neuroretinal rim thickness	Size and orientation of the disc; vascular pattern; parapapillary abnormalities; contour of neuroretinal rim	52.9
J	82	3	6	VCDR; parapapillary atrophy; site of maximum neuroretinal rim thickness (ISNT); size of disc; displacement of vessels not likely to be useful	Arrangement or sectorial location of cupping; vascular pattern	64.7
K	80	5	5	Size of disc; CDR; shape of disc and cup	Size of disc; CDR; shape of cup; contour of neuroretinal rim	64.7
L	80	6	4	Size of disc; CDR; shape of disc; degree of disc tilting; vascular pattern	Diameter of disc; choroidal pattern; vascular pattern; shape of disc	47.1
M	78	8	3	Size and shape of disc; size of cup; vasculature pattern; color of parapapillary region; depth of cup	Size of disc; size of cup; pattern of vessels; depth of cup; parapapillary pigmentation	82.4
N	76	2	10	Size and shape of disc; shape of cup; individual characteristics likely to be less important than overall gestalt of ONH pattern	Pattern of choroid and retinal pigment epithelium; blood vessel pattern; neuroretinal rim width not useful	76.5
O	74	7	6	Size of disc and cup; vessel pattern; color of the optic nerve	Height of the optic nerve relative to retinal plane; vessel pattern; optic disc size more useful than cup size; tilt of disc; parapapillary atrophy	64.7

Data were collected in qualitative questioning before the experiment regarding the most heritable features of the optic nerve head and after the experiment about how the judgments were reached. CDR, cup-to-disc ratio; VCDR, vertical cup to disc ratio; ISNT, inferior>superior>nasal>temporal neuroretinal rim sector thickness.

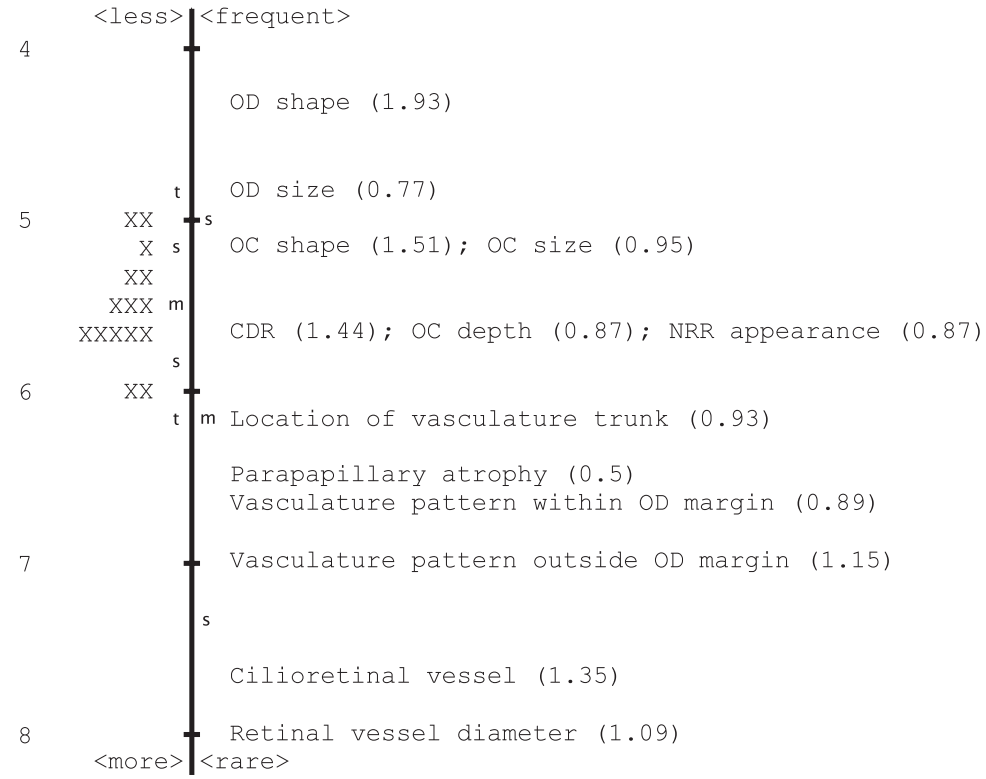

FIGURE 3. Performance of ophthalmic experts and the relative usefulness of specific ONH features in determining zygosity. *Left* of the *vertical line*: graders, represented by X; *right*: ONH items. More able graders and less useful items, for determining zygosity by ONH appearance are near the bottom. The *t*-standardized-information, weighted mean square statistic is displayed in parentheses, with the column of figures on the *left* representing the logit values of the rank-adjusted proportions. s, 1 SD; t, 2 SD; m, mean; OD, optic disc; OC, optic cup; CDR, cup-disc ratio; NRR, neuroretinal rim.

Two of the ophthalmic experts correctly determined zygosity in 45 of 50 twin pairs. The zygosity of 20 (40%) twin pairs was correctly nominated by all 15 experts, whereas the zygosity of 3 (6%) pairs (Fig. 2) was correctly identified by fewer than 5 of the graders.

For each expert and across the panel as a whole, the proportion of incorrect zygosity calls did not differ ($P > 0.1$) between the first and second halves of the viewed set. MZ twins were more frequently erroneously identified as DZ than vice versa (Table 1). The three graders who specified more DZ twins than MZ generally performed poorly on the grading, each with scores less than 84%.

On completion of the experiment, each expert had an altered opinion regarding the traits that he or she believed were heritable, relative to the specific responses in the qualitative initial interview (Table 1). Rasch analysis of the quantitative weighting revealed that experts who correctly identified the zygosity in more than 85% of cases placed the most weighting on the size and shape of the optic disc and optic cup, whereas experts with the lowest scores placed greater weighting on the ONH vasculature (Fig. 3). The empirically adjusted ability of the experts, each represented by an X is displayed, with better-performing experts and less-useful ONH characteristics for determining zygosity appearing near the bottom of the graph. The item optic disc shape had noticeable off-variable noise (infit mean square, 1.93).

During the second experiment, the ONH photographs from the same individual were matched correctly in an average of 64.7% of the attempts (range, 47.1%–82.4%). Across the experts, there was no correlation between the results of the zygosity-nominating experiment and the second ONH-matching experiment (Spearman's rho: −0.16, $P = 0.27$). Two of the three best performing experts in this second experiment also had the greatest disparity in the proportion of twin zygosities incorrectly nominated as MZ or DZ (Table 1).

DISCUSSION

The degree to which genes and environmental factors determine ONH morphology is of fundamental importance for the full understanding of the etiology of common blinding diseases such as glaucoma. We used a novel approach to dissect the heritable features of the ONH. In determining the heritable components, the results of the study suggest that quantification of the shape and size of the optic disc and cup should receive a greater priority than quantification of ONH vascular features. Factors such as vascular pattern or tortuosity are difficult to quantify; hence, our findings are strengthened by the fact that this was a qualitative study rather than a quantitative one. The relative partitioning of genetic and environmental components of particular traits allows for informed investigation of underlying pathogenesis. Given the vast array of potentially quantifiable ONH structures, prioritizing those that should be investigated over others allows for efficient use of finite resources.

The tight coupling of ONH traits and disease underscores the relevance of the genetic liability that is associated with those specific features.[4] Mutations in both nuclear and mitochondrial genes alter ONH architecture in the diseased state.[15–17] Glaucoma, a disease of progressive excavation of the optic disc, for example, has been demonstrated to have a genetic basis.[18–20] It is noteworthy that each expert grader differed in their responses before and after completing the experiment. The initial unstructured questioning allowed exploration of the pre-experiment biases regarding heritable traits of the ONH. Of note, in this initial questioning, optic disc size was regarded as the most important trait by 11 of the 15 experts. The postexperiment questioning gave insight into each grader's intuition on these heritable features. Three of the top five performing experts commented that vascular pattern was not useful in determining twin zygosity, whereas the four poorest performing graders principally used these features in determining the zygosity.

In the second experiment, we investigated the relative phenomenon of mirroring, laterality and environment effects on the ONH. Unique environmental factors and variable expression of genetic factors (e.g., differences in methylation) may account for phenotypic differences in MZ twins.[21] In classic twin studies, the equal-environment assumption is widely accepted,[22] and within individuals there is a degree of ONH asymmetry.[2] However, the ONH features of some MZ twins are surprisingly dissimilar, thereby suggesting that stochastic epigenetic events significantly influence ONH architecture. This phenomenon is the likely reason for the two sets of MZ twins pairs that most experts incorrectly determined as being DZ pairs in the initial experiment. Although the phenomenon of mirror imaging has been described in MZ twins,[23,24] it does not appear to be of marked significance in ONH development. The principal limitation of this second experiment was the relatively small number of MZ twins used. Increasing both the number of expert graders and the number of twins used would allow for a clearer demarcation of traits commonly discordant between MZ twins and the possible identification of novel factors influencing the ONH appearance.[25]

Populations of twins provide a powerful opportunity for disentangling complex genetic and environmental interactions.[22] A classic twin study allows analysis of the variance and covariance between MZ and DZ twin pairs. Comparison between the covariance of MZ and DZ twin pairs allows partitioning into dominant versus additive genetic components and shared versus nonshared environmental elements.[22] A small number of low-powered twin studies have been conducted to investigate the ONH ,and these generally support the results of our findings. Twins were included in the cohort used by Armaly[26] in his landmark paper in which he concluded that the CDR of the ONH was genetically determined. Subsequently, Schwartz et al.[27,28] used ONH photographs from twins to estimate that the heritability of the CDR ranges between 70% and 80%. Teikari and Airaksinen[29] also identified greater CDR correlations between MZ twins than between DZ pairs. The parapapillary retinal nerve fiber layer thickness has been found to have a relatively high heritability (82%), whereas the presence of cilioretinal arteries are influenced by additive genetic factors, with an estimated heritability of 71%.[30,31] Huntzinger and Christian[32] concluded that vascular tortuosity is likely to be more genetically determined than other features, such as vessel length, branching points, and number of vessels crossing the optic disc margin. Although family-based studies investigating ONH heritability have been focused on few phenotypic features, the findings also generally support the overall results of our study. The size of optic cup and disc[33–35] have been found to be more hereditable than has retinal vessel thickness.[36]

In summary, Rasch analysis demonstrated that both the shape and size of the optic disc and cup were more useful in determining twin zygosity than were vascular parameters. Thus, these traits are particularly likely to be highly heritable. Nonetheless, epigenetic variation causes minor asymmetry of the ONH. Determining the genetic and environmental variants that influence ONH morphology will allow for the elucidation of the molecular pathogenesis of diseases that alter optic nerve architecture.

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