The Path to Open-Angle Glaucoma Gene Discovery: Endophenotypic Status of Intraocular Pressure, Cup-to-Disc Ratio, and Central Corneal Thickness

Jac Charlesworth,[1] Patricia L. Kramer,[2,3] Tom Dyer,[1] Victor Diego,[1] John R. Samples,[4] Jamie E. Craig,[5] David A. Mackey,[6,7,8] Alex W. Hewitt,[9] John Blangero,[1] and Mary K. Wirtz[4]

Purpose. Primary open-angle glaucoma (POAG) is a complex disease with a genetic architecture that can be simplified through the investigation of individual traits underlying disease risk. It has been well studied in twin models, and this study was undertaken to investigate the heritability of some of these key endophenotypes in extended pedigrees.

Methods. These data are derived from a large, multicenter study of extended, Caucasian POAG families from Australia and the United States. The study included 1181 people from 22 extended pedigrees. Variance components modeling was used to determine the heritabilities of maximum intraocular pressure (IOP), maximum vertical cup-to-disc ratio (VCDR), and mean central corneal thickness (CCT). Bivariate quantitative genetic analysis between these eye-related phenotypes and POAG itself was performed to determine whether any of these traits represent true endophenotypes.

Results. Heritability estimates for IOP, VCDR, and CCT (0.42, 0.66, and 0.72, respectively) were significant and show strong concordance with data in previous studies. Bivariate analysis revealed that both IOP (RhoG = 0.80; $P = 9.6 \times 10^{-6}$) and VCDR (RhoG = 0.76; $P = 4.8 \times 10^{-10}$) showed strong evidence of genetic correlation with POAG susceptibility. These two traits also correlated genetically with each other (RhoG = 0.45; $P = 0.0012$). Alternatively, CCT did not correlate genetically with risk of POAG.

Conclusions. All the proposed POAG-related traits have genetic components. However, the significant genetic correlations observed between IOP, VCDR, and POAG itself suggest that they most likely represent true endophenotypes that could aid in the identification of genes underlying POAG susceptibility. CCT did not correlate genetically with disease and is unlikely to be a useful surrogate endophenotype for POAG. (*Invest Ophthalmol Vis Sci.* 2010;51:3509–3514) DOI:10.1167/iovs.09-4786

Glaucoma is a major cause of visual impairment and the second leading cause of blindness worldwide.[1] The most common form is adult-onset primary open-angle glaucoma (POAG), which has a strong genetic component, with family history of the disease an acknowledged risk factor.[2–4] A 10-fold increase in risk of POAG has been documented in first-degree relatives of affected individuals,[3] whereas underreporting of family history suggests that the genetic component of POAG is even greater than is generally acknowledged.[5]

POAG is not a single disease. It comprises many heterogeneous disorders that have yet to be systematically defined. The clinical diagnosis of glaucoma is based on a combination of several main features, including specific changes in the appearance of the optic nerve head constituting glaucomatous optic neuropathy, characteristic visual field loss with a slow and often asymptomatic progression,[6] and in most cases increased intraocular pressure (IOP). Central corneal thickness (CCT) has also been suggested as a risk factor for POAG.[7–10] Evidence suggesting that POAG is a group of disorders rather than a single entity includes the mapping of multiple major POAG loci primarily representing Mendelian forms of the disease,[11–18] broad phenotypic heterogeneity,[19,20] and differences in response to treatment.[21,22]

Susceptibility to complex disorders, particularly late-onset diseases such as glaucoma, results from the combined interaction of genes and environmental factors. Dissecting the genetic architecture of these heterogeneous disorders is best achieved by considering individual quantitative traits or endophenotypes underlying the disease.[23] Quantitative endophenotypes allow individuals to be ranked along the continuum of risk, thus providing substantially more information than dichotomous measures of affection status. Among the established risk factors for POAG, IOP,[24] optic nerve cupping as measured by cup-to-disc ratio (CDR),[7] and CCT[24,25] represent plausible endophenotypes. These traits are state-independent and have shown evidence of heritability in twin studies,[26–32] sibships and small pedigrees,[33–35] and isolated populations.[36] However, studies of heritability in large, extended pedigrees, which generally produce the most accurate estimates of heritabilities due

From the [1]Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, Texas; the Departments of [2]Neurology and [3]Molecular and Medical Genetics and the [4]Casey Eye Institute, Department of Ophthalmology, Oregon Health and Science University, Portland, Oregon; the [5]Department of Ophthalmology, Flinders University, Adelaide, South Australia, Australia; the [6]Centre for Ophthalmology and Visual Science, University of Western Australia and [7]Lions Eye Institute, Perth, Western Australia, Australia; the [8]Discipline of Medicine, University of Tasmania, Hobart, Tasmania, Australia; and the [9]Centre for Eye Research Australia, University of Melbourne, Melbourne, Victoria, Australia.

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Corresponding author: Mary K. Wirtz, Department of Ophthalmology, Oregon Health & Sciences University, 3375 SW Terwilliger Boulevard, Portland, OR 97201; wirtzm@ohsu.edu.

to their relative insensitivity to latent shared environmental factors, are currently very limited.[37]

Regardless of the availability of quantitative risk factors that correlate phenotypically with POAG risk, a critical requirement for an endophenotype is the establishment of genetic correlation between the endophenotype and the disease itself. Mere phenotypic correlation between disease and risk factor does not guarantee that the genetic analysis of such a risk factor will enhance the search for disease-related genes. Genetic correlation, however, specifically measures the magnitude of shared genetic determination between two traits (in this context, between the putative endophenotype and the focal disease risk). Without significant genetic correlation, dissection of genetic factors for a quantitative risk factor will not enhance the search for genes involved in disease pathogenesis. To determine the value of IOP, CDR, and CCT as potential endophenotypes of POAG, we performed a quantitative genetic study of large extended families.

METHODS

Ethics Approval and Consent

Ethics approval was obtained from the Oregon Health and Science University (OH&SU) Institutional Review Board (Portland, OR), the Human Research Ethics Committees of the Royal Victorian Eye and Ear Hospital (Victoria, Australia), and the Human Research Ethics Committee (Tasmania) Network. This study was conducted in accordance with the tenets of the Declaration of Helsinki. Written, informed consent was obtained from all participants.

Study Populations

This project was a collaboration of the Oregon Glaucoma Genetics Study (OGGS)[12,15] and the Glaucoma Inheritance Study in Tasmania (GIST).[38] The OGGS consists of extended POAG pedigrees from the Northwestern United States, collected through the Casey Eye Institute at OH&SU. GIST is a large population study of glaucoma families based in Australia, primarily from the island state of Tasmania. All participants from both studies were of Caucasian descent. The study population consisted of 570 men and 611 women. The average age of the clinically investigated participants was 53.7 ± 18.9 years.

The study included 16 pedigrees from the OGGS, including a total of 700 individuals, 421 with clinical information. The average pedigree size was 44 individuals, with the largest containing 151 individuals. There were six pedigrees included from the GIST, comprising a total of 481 individuals, 231 with available clinical information. The average pedigree size was 80 individuals, with the largest including 139 individuals. The total study population was therefore composed of 22 families including 1181 individuals, of whom 652 provided clinical data. These pedigrees do not represent the Mendelian form of POAG, but were collected by ascertainment of POAG probands and extrapolated to include all affected and unaffected relatives. A complete listing of the families included in the study is shown in Table 1.

TABLE 1. Summary of the Pedigrees

Population	Pedigree ID	Total Individuals	Clinically Assessed	Clinically Diagnosed
United States	93001	132	78	22
	94001	27	20	10
	94004	43	24	11
	94005	26	21	8
	94007	13	7	5
	95001	19	9	6
	95002	60	40	14
	95003	16	12	4
	96001	40	22	8
	96004	15	12	4
	97001	22	12	4
	97006	37	15	13
	98002	151	91	21
	98003	55	30	11
	99001	23	13	9
	99011	21	15	5
Australia	GTas03	139	64	31
	GTas04	85	38	20
	GTas11	25	8	6
	GTas17	77	37	12
	GTas37	105	54	35
	GTas54	50	30	14
Total	22 Pedigrees	1181	652	273

Clinical Examination

Clinical examination and diagnosis of patients involved in the GIST and OGGS have been documented previously.[12,15,39,40] In brief, POAG was clinically defined as an optic neuropathy which exhibits optic nerve head excavation with thinning of the neuroretinal rim, often with Drance-type nerve fiber layer hemorrhages; notching; pitting; significant focal loss or general loss of retinal fiber layer (generally measured by an enlarged vertical cup-to-disc ratio [VCDR] ≥0.7); and visual field defects consistent with the disc changes and with common descriptions of glaucomatous field loss.[15,39] Glaucoma cases secondary to trauma or anterior segment dysgenesis were excluded. Quantitative trait measures (IOP, VCDR, and CCT) were collected as part of the clinical examination of POAG patients and their relatives, and most were collected across multiple time points.

IOP was assessed with Goldmann applanation tonometry with a recently calibrated tonometer. IOP measurements were obtained by slit lamp, with the subjects in upright position. A combined anesthetic and fluorescein product was used before pressure determination. Most of the subjects had undergone several examinations over the past 15 years. In addition, we obtained medical records from all individuals with diagnosed POAG, to obtain premedication IOP data. In general, multiple IOP measures were available for each individual; hence, maximum IOP measured by Goldmann applanation across all records was selected as the endophenotype of interest, to further reduce any bias introduced by postmedication IOPs. Age at examination was always matched to the selected IOP measure. No corrections were made for corneal thickness, since this endophenotype was investigated simultaneously.

Optic disc appearance was classified by one to two clinicians at the time of examination with a slit lamp biomicroscope after pupil dilation. Stereo fundus photographs were obtained with a portable fundus camera after dilation (Genesis; Kowa-Optimed Inc., Torrance, CA) for future reference. When there was a discrepancy between the two examiners, the stereo disc photos were independently assessed by a third glaucoma specialist. Establishing the size of the optic nerve head is considered essential in evaluating cupping. The sizes of the cups were evaluated by using the standard method of a reference spot from a direct ophthalmoscope or measurements in the dilated eye with a 78-D lens. Optic nerve characteristics of interest included vertical and horizontal CDRs, vessel displacement, and pallor of the neuroretinal rim, with respect to focal notching and depth of the cup; however, the larger VCDR in either eye was used as the trait measure.

CCT was assessed by ultrasound pachymetry (Pachmate DGH 55; DGH Technology, Exton, PA) with 25 measurements of each eye. The pachymeter was calibrated according to the manufacturer's guidelines before the start of the clinic. The corneal velocity was set at 1640 m/s, which is the currently accepted value for the speed of sound through the cornea. The subject was placed in the examination chair, and the anesthetic (0.4% proparacaine) was administered to both eyes. The average of the 25 measurements was recorded along with the time of day. Confounding factors for establishing CCT include excimer laser refractive surgery and long-term use of hard contact lenses. CCTs in

TABLE 2. Heritability Measures of the POAG Related Traits Including Significant Covariates

Trait	Heritability	*P*	SE	Significant Covariates	Variance due to Covariates	Typed Individuals (*n*)
POAG diagnosis	0.812	6.0×10^{-7}	0.173	Age, age–sex	Not calculated	648
Normalized maximum IOP	0.417	6.4×10^{-10}	0.084	Age, age–sex	5.8%	630
Maximum VCDR	0.658	9.4×10^{-19}	0.083	Age, population	24.0%	597
Normalized mean CCT	0.721	5.0×10^{-7}	0.157	Age, age–sex, sex, population	16.7%	215

individuals who had undergone laser surgery or wore hard contact lenses were eliminated completely from the analysis.

Statistical Methods

Heritability was estimated with maximum-likelihood–based variance-component modeling in SOLAR 4.2,[41] taking into account all pedigree relationships simultaneously. Traits showing severe non-normal distributions, such as high kurtosis, were directly normalized with an inverse normal transformation to eliminate distribution errors and reanalyzed. Age, sex, and their interactions were included as covariates in all analyses. An additional covariate assigning pedigrees to either the Australian or North American population was also included, to account for potential population differences. A likelihood ratio statistic was used to test whether each estimated heritability was significantly different from 0. The resultant test statistic is distributed as a 50:50 mixture of a χ^2 distribution with 1 *df* and a point mass at 0.

We also examined the sources of covariation between these traits by using bivariate quantitative genetic analysis, as implemented in SOLAR,[42] to assess the genetic correlations between these eye-related phenotypes and POAG itself, to determine whether any of these traits represent true endophenotypes that may aid in the search for pleiotropically acting genes directly related to disease risk. For the joint analysis of POAG (a dichotomous trait) and each of the potential quantitative endophenotypes, we used the extension of the threshold liability model described by Duggirala et al.[43] and implemented in SOLAR. In this model, the phenotypic correlation between two traits is assumed to be a function of the genetic and environmental correlations between the traits. Using this strategy, we first tested whether there was evidence of phenotypic correlation between any pair of traits by using a likelihood ratio test that explicitly allows for nonindependence among related individuals. In addition, we tested whether a component of the phenotypic correlation was due to the effect of genes acting jointly on both traits, by comparing a model in which all correlation parameters are estimated against a model in which the genetic correlation (RhoG) is constrained to 0. The detection of a genetic correlation between two traits that is significantly greater than 0 is interpreted as evidence of pleiotropy (i.e., evidence of genes that jointly influence both traits). Conventional likelihood-ratio statistics were used to test whether observed estimated correlations were significantly different from 0.

Information on genetic correlations is drawn from the cross-correlations between traits and relatives. It reflects those genetic effects that act jointly on the two traits under consideration. In the absence of genetic factors (and shared environmental factors), there is expected to be no correlation between, say, a father's CCT and his son's IOP. All pedigree information is properly used (and weighted) to obtain this estimate of genetic correlation. The phenotypic correlation is obtained from the sum of standardized genetic covariances and environmental covariances for a given pair of traits. The phenotypic correlation is the most common type of correlation reported in the literature; however, with nonindependent data the phenotypic correlation must be estimated with a maximum-likelihood procedure as opposed to the standard Pearson correlation method.

RESULTS

Clinical Diagnosis of POAG

There were 648 individuals with available clinical diagnosis data. In total, there were 273 individuals with POAG across all pedigrees (Table 1). The heritability of POAG affection status was estimated at 0.81 ± 0.17 ($P = 6.0 \times 10^{-7}$), shown in Table 2. Age and age–sex interaction were both significant covariates.

Intraocular Pressure

There were 630 individuals with maximum IOP and age at examination recorded in the study population. The mean trait value was 19.6 ± 5.5 mm Hg but the observed distribution was leptokurtic (kurtosis = 3.47; Table 3). Since frank deviations from normality can have detrimental effects on variance component estimation, we used an inverse normal transformation of the data. This procedure is conservative with regard to the detection of genetic effects. The heritability of maximum IOP was determined to be 0.42 ± 0.08 ($P = 6.4 \times 10^{-10}$) as shown in Table 2. Age and age–sex interaction were both significant covariates, accounting for 5.8% of the total trait variance.

Vertical Cup-to-Disc Ratio

There were 597 individuals with maximum VCDR and age-at-examination data in the study. The mean trait value was 0.47 ± 0.25, with a relatively normal distribution requiring no additional transformation (Table 3). The heritability of maximum VCDR was 0.66 ± 0.08 ($P = 9.4 \times 10^{-19}$) as shown in Table 2. Age and population of origin were the only significant covariates, accounting for 24% of the variance in VCDR.

Central Corneal Thickness

The study included 215 individuals with CCT measures. The trait mean was 463.0 ± 41.1 μm and showed evidence of non-normal distribution with high kurtosis (Table 3). Therefore, an inverse normal transformation was applied. Mean CCT had a heritability of 0.72 ± 0.16 ($P = 5.0 \times 10^{-7}$) as shown in

TABLE 3. Trait Distributions for the POAG Related Traits

Trait	Mean	Minimum	Maximum	SD	Skewness	Kurtosis
Maximum IOP	19.64	8.00	46.00	5.47	1.69	3.93*
Maximum VCDR	0.47	0.10	1.0	0.25	0.41	−1.05
Mean CCT	565.7	463.0	732.5	41.10	0.60	1.46*

* Kurtosis too high, normalization of trait is necessary.

TABLE 4. Bivariate Modeling Results

POAG Related Traits		Genetic Correlation	*P*	Phenotypic Correlation	*P*
POAG diagnosis	Maximum IOP	**0.801**	$\mathbf{9.6 \times 10^{-6}}$	**0.552**	$\mathbf{6.0 \times 10^{-21}}$
POAG affected	Maximum VCDR	**0.761**	$\mathbf{4.8 \times 10^{-10}}$	**0.672**	$\mathbf{1.2 \times 10^{-30}}$
POAG affected	Mean CCT	−0.280	0.266	**−0.250**	**0.029**
Maximum IOP	Maximum VCDR	**0.445**	$\mathbf{1.2 \times 10^{-3}}$	**0.292**	$\mathbf{5.5 \times 10^{-11}}$
Maximum IOP	Mean CCT	0.031	0.891	0.011	0.896
Maximum VCDR	Mean CCT	**−0.470**	$\mathbf{2.1 \times 10^{-2}}$	**−0.242**	$\mathbf{3.7 \times 10^{-3}}$

Significant correlations are shown in bold; maximum IOP and mean CCT were normalized before bivariate modeling.

Table 2. Age, sex, age–sex interaction, and population of origin were all significant covariates, accounting for 16.7% of the trait variance.

Tests for Pleiotropy Identify Quantitative Endophenotypes

For each quantitative trait, we used bivariate quantitative genetic analysis to test for evidence of the pleiotropy effects of genes that have effects on both POAG risk and the quantitative trait. A significant genetic correlation between disease risk and a quantitative trait is a mandatory prerequisite for recognition of the trait as an endophenotype for disease. Table 4 shows the results of these formal tests of endophenotypic potential. The analysis of POAG and IOP revealed a highly significant positive phenotypic correlation (RhoP = 0.55; $P = 6.0 \times 10^{-21}$) which is driven by significant evidence of genetic correlation (RhoG = 0.80; $P = 9.6 \times 10^{-6}$). Thus, approximately 64% of the genetic variation in POAG risk and quantitative IOP levels was due to shared pleiotropically acting genes. Similarly, POAG and VCDR exhibited significant phenotypic correlation (RhoP = 0.67; $P = 1.2 \times 10^{-30}$) induced primarily by genetic correlation (RhoG = 0.76; $P = 4.8 \times 10^{-10}$). Thus, both IOP and VCDR showed evidence of strong genetic and phenotypic correlations with clinical diagnosis of POAG and as such are solid POAG endophenotypes. However CCT showed no evidence of a significant genetic correlation (RhoG = −0.28; $P = 0.27$) but some evidence of a negative phenotypic correlation (RhoP = −0.25; $P = 0.029$) with clinically assessed POAG status. This lack of evidence of a substantial genetic overlap between POAG risk and CCT suggests that this trait, although a phenotypically correlated risk factor of POAG, does not represent an endophenotype that could enhance the search for POAG-related genes.

To determine whether IOP and VCDR represent independent endophenotypes, we also performed bivariate quantitative genetics among the quantitative traits. There was strong evidence of a positive phenotypic correlation between IOP and VCDR (RhoP = 0.29; $P = 5.5 \times 10^{-11}$) and some evidence that the correlation may be due to shared genetic effects (RhoG = 0.45; $P = 1.2 \times 10^{-3}$) as shown in Table 4. However, the magnitude of the genetic correlation implies only incomplete genetic overlap between these two endophenotypes. Thus, studies focused on genetic dissection of POAG could use both of these traits without fear of genetic redundancy. There was also evidence of a negative correlation between the VCDR and CCT endophenotypes (RhoP = −0.24; $P = 3.7 \times 10^{-3}$) and some evidence of shared genetic effects (RhoG = −0.47; $P = 2.1 \times 10^{-2}$) as shown in Table 4. Therefore, there is evidence of shared genetic effects acting jointly on VCDR and CCT. However, given the lack of genetic correlation of CCT with POAG risk, it appears that this observation reflects the actions of genes unlikely to be involved in POAG directly. There was no evidence of either genetic or phenotypic correlation between IOP and CCT (Table 4).

DISCUSSION

POAG is clearly a complex disease and more likely represents a heterogeneous group of eye disorders. There has been minimal success to date in mapping POAG genes by using disease diagnosis as the trait. The POAG loci identified so far represent simple Mendelian forms of the disorder, and mutations in these genes are relatively rare. Most cases of glaucoma result from complex combinations of genetic and environmental factors; thus, mapping susceptibility genes for these multifactorial cases is not simple. One method of resolving the genetic components in complex diseases is to focus on underlying quantitative traits, or endophenotypes, that are associated with the disease, but by themselves do not cause the disease. Several criteria define an endophenotype.[23] It must be heritable, associated with a disease in the general population, and primarily state-independent (the trait manifests in individuals regardless of disease state). Finally, in families with the disease, high (or low) levels of the trait must occur in unaffected family members at a higher rate than in the general population. More formally, the latter condition implicitly requires that disease risk be correlate genetically with the endophenotype. Therefore, endophenotypic status requires both heritable, state-independent variation of the quantitative trait and genetic correlation between the trait and disease. The genetic overlap documented by a significant genetic correlation indicates that genes found in the dissection of an endophenotype are more likely to be involved in disease risk. Endophenotype-based methods have been successfully used to map genes for a variety of disorders.[44–48]

Extended pedigrees generally provide the most accurate estimates of additive genetic heritability (i.e., cumulative effect of individual genes) since they are less susceptible to confounding due to shared environment, because related individuals cross in many different households. Accurate estimates of additive genetic heritability provide the fundamental rationale for searching for specific causal genes. In addition, such a heritability estimate provides a target for the extent of phenotypic variation that ultimately could be explained given information on complete genetic sequence variation. Our heritability estimate for IOP (0.41 ± 0.08) is well within the range of those in previous studies, with studies of sibships and smaller pedigrees showing lower estimates (0.29–0.36),[34,35,49] whereas twin studies showed slightly higher estimates for IOP (0.62–0.67),[31,32] due to the effects of shared environment. Our estimate for the heritability of CDR (0.66 ± 0.08) again fell between the previous estimates for sibs (0.48–0.56)[34,35] and twins (0.79).[27] CCT has shown high heritability estimates in twin studies (0.88–0.95),[28,29] whereas a more recent study of nuclear families produced a heritability estimate of 0.68.[33] Our heritability estimate for CCT in extended pedigrees (0.72 ± 0.16) was again well between these two alternative study designs. Population of origin was a significant covariate in the modeling of VCDR and CCT; however, when we analyzed the

two populations independently, there were no major differences in the heritabilities or magnitude of their significance between the two populations for any of the traits (data not shown).

Our bivariate genetic analyses have revealed that both IOP and VCDR represent potentially useful endophenotypes for the genetic dissection of POAG risk. Both of these traits exhibit significant genetic correlations with disease risk, suggesting substantial overlap of genes that jointly influence variation in these quantitative phenotypes and disease status. IOP and VCDR appeared to be not only genetically correlated with POAG but also with each other (RhoG = 0.45; $P = 0.0012$). Although these genetic correlations are indicative of pleiotropy, they do not provide any causal or directional information.

CCT is a recently suggested POAG risk factor,[7,8] but its involvement in POAG pathogenesis has not been clearly determined. Surprisingly, our results failed to support endophenotypic status for CCT, which did not show significant genetic correlation with disease risk. These results may be due to a lack of power, since measurement of this trait was included only in the routine clinical examination of these two populations in the past few years. Hence, there are not as many individuals with CCT measures in this study compared with IOP or VCDR data, which had been measured multiple times across the past decade. Even with the smaller dataset, however, CCT still showed significant evidence of heritability, as well as phenotypic and genetic correlation with VCDR. Based on the results of this study, CCT is not genetically correlated with clinical POAG status and as such is not a true glaucoma endophenotype. Although lack of significance is always partly a function of sample power, at the least, our results suggest that IOP and VCDR are better endophenotypes for POAG risk than is CCT. CCT is, however, a strong trait for genetic analysis since the high heritability indicates that there is a large degree of genetic control over this trait.

Inclusion of quantitative correlates or POAG-related endophenotypes in the genetic analysis of POAG may increase the power to dissect the genetic architecture of this complex disease. The discovery of genes influencing IOP, CDR, and/or CCT may provide significant insights into glaucoma pathophysiology as a whole. However, the field has been relatively slow to switch from searching for "POAG genes" to searching for genes influencing POAG phenotype components. To date, linkage analysis of the individual POAG-related endophenotypes has been limited. IOP is the most widely investigated. In 2005, we identified a locus for IOP on 10q22 in a single, four-generation Australian POAG pedigree.[37] Duggal et al.[50] identified another IOP-related QTL on 19p13 in the Beaver Dam Eye Study. Rotimi et al.[44] identified suggestive evidence of linkage for IOP at 5q22 and 14q22 in sib pairs from West Africa. The lack of concordance between these studies is most likely due primarily to heterogeneity of the genetic control of these traits as well as population variation.

The results of this analysis suggest that using the quantitative endophenotypes IOP and VCDR would more effectively allow us to identify genes relating to POAG susceptibility. Both are significantly heritable, are genetically and phenotypically correlated with disease, and show only mild evidence of shared genetic effects, suggesting that although there may be some pleiotropy, in general, different genes influence each trait. Although CCT may not be a suitable trait for directly identifying POAG-susceptibility genes, it is clearly under strong genetic control. The identification of genes influencing CCT may become more important once the relationship between CCT and POAG is fully elucidated.

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