Major Genetic Effects in Glaucoma: Commingling Analysis of Optic Disc Parameters in an Older Australian Population

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PURPOSE. To test the hypothesis that there is a major genetic determinant of vertical disc diameter (VDD) and vertical cup-to-disc ratio (VCDR) in a large, population-based sample.

METHODS. Data were collected from 3654 individuals, 49 years of age or older, participating in the Blue Mountains Eye Study. VDD and VCDR were determined from stereo optic disc photographs. Commingling analyses in SKUDRIVER/SKUMIX were performed in nonglaucomatous eyes to investigate whether the observed VDD and VCDR data were best described by a one-, two-, or three-distribution model.

RESULTS. VDD data did not show evidence of commingling. After adjustment for the effects of age, VDD and intraocular pressure, the best model for VCDR consisted of a mixture of three distributions in Hardy-Weinberg equilibrium. The proportion of the variance in VCDR explained by this mixing component was 0.58.

CONCLUSIONS. Findings from this study are consistent with the presence of a major gene that accounts for 58% of the variance in VCDR. These results strongly support further efforts to identify the genetic variants responsible for this quantitative trait, which is a key constituent of the phenotype of primary open-angle glaucoma (POAG). (Invest Ophthalmol Vis Sci. 2009;50:5275–5280) DOI:10.1167/ iovs.08-3065

Primary open-angle glaucoma (POAG) is a progressive optic neuropathy with an established genetic component to its origin.1–3 In the majority of cases, POAG is inherited as a complex disease: it is assumed to result from many interactive genetic and environmental factors, none of which individually is necessary or sufficient to cause the disease. Although high-throughput genotyping technologies are becoming increasingly feasible and affordable, and underlying methodologies to unravel complex diseases are developing rapidly, the multifactorial etiology of POAG is still proving a hard nut to crack. More than 25 chromosomal regions have been linked to the disease, but only three genes (MYOC,4 OPTN,5 and WDR366) have been identified. These genes most likely contribute to the pathogenesis of POAG in less than 5% of cases in the general population.7–10 Genes accounting for a greater proportion of the known heritable component of POAG thus remain to be identified.

The etiologic complexity of POAG can be reduced by separately studying quantitative features of the phenotype, such as the vertical optic disc diameter (VDD) or the vertical cup-to-disc ratio (VCDR). Elucidating the genetic determinants of these quantitative features in healthy eyes may improve our understanding of any damage to the optic disc in glaucomatous eyes. Quantitative traits are likely to be more powerful in detecting new genes than the dichotomous POAG trait. They also may have simpler genetic backgrounds, can be studied in entire populations, and are less prone to misclassification.

Obviously, a prerequisite of considering quantitative POAG traits for gene-finding studies is that these traits have a genetic basis. Previous estimates of the heritability of VDD or disc area ranged from 0.52 to 0.73.11–13 Heritability estimates of VCDR ranged from 0.48 to 0.65.12,14 These estimates were based on family studies and provided information on the additive effects of all involved genes. Although a high heritability itself may already be promising for gene-finding studies, it would be interesting to know whether the heritable component solely involves genes of small effect or also includes one or more genes that have a relatively large effect. The latter would be easier to detect in gene-finding studies, and their presence would therefore even more resolutely support quantitative trait–based strategies.

To date, no investigation into possible major genetic effects on VDD and VCDR has been made. A suitable method of examining the population distribution of a quantitative trait for major genetic effects is the use of commingling analysis,15 a form of model fitting that employs the method of maximum likelihood.16 Commingling analysis investigates the strength of evidence for a single gene of major effect and provides an estimate of the locus-specific heritability, which is the proportion of the total phenotypic variance explained by the effect of the major gene.

To explore the feasibility of applying VDD and VCDR to population-based gene-finding strategies, we performed a commingling study on optic disc data from the Australian Blue Mountains Eye Study cohort. We investigated the population distribution of VDD and VCDR for major genetic determinants and estimated their locus-specific heritability.
METHODS

Study Population
The Blue Mountains Eye Study (BMES) is a population-based survey of vision and common eye diseases in the Blue Mountains region west of Sydney, Australia. The study adhered to the tenets of the Declaration of Helsinki and was approved by the Western Sydney Area Health Service Human Ethics Committee. Written, informed consent was obtained from all participants. The population has been described in detail elsewhere. In brief, all permanent noninstitutionalized residents 49 years of age or older were invited to participate. Of the 4435 eligible individuals, 3654 (82.4%) attended baseline eye examinations between 1992 and 1994. Of the 779 nonparticipants, 501 (11.3%) refused, 68 (1.5%) had died, and 210 (4.8%) had moved away from the area. The response rate compares well with the best population-based research in glaucoma.

Clinical Examination and Optic Disc Grading
All subjects underwent comprehensive eye examinations, including assessment of subjective refraction with a logMAR chart, and measurement of intraocular pressure (IOP) with Goldmann applanation tonometry. Visual fields were initially assessed with a 30° suprathreshold screening test (Humphrey 76-point test; Carl Zeiss Meditec, Inc., Dublin, CA). Full-threshold 30-2 visual field tests of each eye were subsequently performed in subjects with suspected glaucoma.

After pupil dilation, 30° color stereoscopic optic disc photographs were taken with a fundus camera (model FF5; Carl Zeiss Meditec, Inc.). Slide transparencies (35 mm) were mounted in clear plastic sheets. Optic disc parameters were assessed with a Donaldson stereo viewer with a template of small circles (Pickett circles number 1203) placed under one of the stereo pair, as described and validated previously. The vertical disc diameter (VDD) was measured to the nearest 0.01 mm as the longest diameter between the inner limits of the scleral ring in a range between clock hours 11 to 1 and 5 to 7. The optic cup was determined by its contour, with the outer margin taken to be the point where the wall met the plane of the disc surface at the level of the scleral ring. The vertical cup-disc ratio (VCDR) was calculated from the disc and cup measurements. Optic disc measurements were corrected for the magnification effect of the eye-camera system according to spherical equivalent refraction, as described by Bengtsson and Krakau. All photographs were graded by one or both of two trained graders. The chief investigator (PM) adjudicated discrepancies. Interobserver variability was assessed in a masked fashion in a random sample of 100 optic discs and was in the excellent agreement range.

Selection Criteria
Because the magnification correction for optic disc measurements used in this study is inaccurate after cataract surgery, subjects who were aphakic or pseudophakic in both eyes were excluded from analyses (n = 108). If only one eye of a subject was phakic, this eye rather than the nonphakic fellow was considered for analysis. If both eyes were phakic, one eye was chosen at random for inclusion in the analysis. Eyes with tilted optic discs (n = 78) or with other disc anomalies, such as colobomata (n = 1), disc drusen (n = 1), or optic atrophy (n = 1), were excluded from the dataset. A further 16 eyes were excluded because of high myopia (spherical equivalent greater than −8 D). The main analyses in this study were performed on a “normal” population, which excluded patients with glaucoma in either eye (n = 90). The diagnosis of glaucoma was made on the basis of typical glaucomatous visual fields loss on the Humphrey 30-2 test, combined with matching optic disc rim thinning, as described previously. For the analyses of VDD, 86 eyes were excluded because no gradable optic disc photographs were available, and for the analyses of VCDR, an additional 5 eyes were excluded because covariate data were incomplete, leaving valid data for 3273 and 3268 subjects for the analyses of VDD and VCDR, respectively.

Statistical Analysis
Before the commingling study, univariate and multivariate linear regression analyses were performed (SPSS ver. 11.5 for Windows; SPSS, Chicago, IL) to detect whether any adjustments were needed for the effects of explanatory covariates. Putative covariates of VDD and VCDR that were studied included age, sex, height, history of migraine, intraocular pressure (IOP), and (for VCDR analysis only) VDD. Adjusted VDD and VCDR data were standardized to have a mean of 0 and a variance of 1.

Commingling analysis investigates whether the observed distribution of a quantitative trait is best modeled by a single distribution or by an admixture of multiple distributions. The latter could indicate that a gene of major effect underlies the trait. If the major gene has an allele frequency of q; genotypic means of m1, m2, and m3; and within-genotype variance s2, a likelihood function L for an individual observation is defined under Hardy-Weinberg equilibrium as:

\[
L(q, m_1, m_2, m_3; s^2; x) = q^2 f(x; m_1, s^2) + 2q(1-q)f(x; m_2, s^2) + (1-q)^2 f(x; m_3, s^2)
\]

where x is the observed trait value of a randomly ascertained member of the population and f(x; m, s^2) is a normal density function with mean m and variance s^2. The overall likelihood of this mixture model is computed as the product of the likelihoods of the individual observations. The maximum likelihood of the model may be compared with that of the null model, which consists of a single normal distribution, for a test of the major gene effect.

The software used to implement the commingling analysis was the C++ program SKUDRIVER, written by one of the authors (ACV), and the program SKUMIX. Both programs are available at http://statgen.iop.kcl.ac.uk/skudriver/. The commingling analysis was performed on the adjusted and standardized VDD and VCDR data, and comprised maximum likelihood estimation for each of three models: single-distribution (the null model), two-distribution (i.e., fully dominant or recessive) and three-distribution model.

SKUDRIVER takes as input a user-specified range of starting values for each of the following variables (Fig. 1): within-genotype variance (V), homozygote mean (U), dominance (D), displacement (T), allele frequency (Q), power transform variables (P and R), and inbreeding
coefficient (F). Displacement (T) is defined as the difference between the mean values of the two homozygote distributions. Dominance (D) represents the mean value of the heterozygote distribution relative to the two homozygotes. Thus, the three genotypic means are at U, U + DT, and U + T. Since the input parameters in SKUDRIVER can be specified as either “fixed” or “estimated,” the user may constrain the model to a single distribution by fixing the value of T as 0, or may specify a two-distribution model by fixing the value of D as 0 or 1. Q is assigned to be the frequency of the allele associated with the displaced distribution so that in the three-distribution model, under Hardy-Weinberg equilibrium, the proportions of the population within each of the distributions are (1 − Q)², 2Q(1 − Q), and Q². However, the program also allows deviation from Hardy-Weinberg equilibrium by introducing an inbreeding coefficient F, so that the proportions within the distributions become (1 − Q)² + FQ(1 − Q), 2Q(1 − Q)(1 − F), and Q² + FQ(1 − Q).27

One of the important features of the software is the facility to specify starting values for the parameters in SKUDRIVER. In this way a grid search of the likelihood surface is conducted, minimizing problems of singularities or local maxima.28 The SKUMIX program provided a measure of the goodness of fit for the one-, two-, and three-distribution models, both with and without a power transformation, expressed as minus twice the logarithm of the likelihood (−2 log L). Hypothesis testing was achieved by referring the difference in this quantity between two models to a χ² distribution with degrees of freedom equal to the difference in the number of free parameters. Since multiple comparisons were made, each probability was corrected with a Bonferroni correction, to avoid spuriously significant results.29 The best-fitting model was chosen according to the Akaike Information Criterion (AIC), defined as −2 log L + twice the number of free parameters.30 The AIC penalizes for adding free parameters and thus selects the most parsimonious model that fits the data well. The Akaike weight (w) was used to assess model selection uncertainty.31 It represents the probability that the model is the best among the whole set of models.

RESULTS

Demographic and ophthalmic characteristics of the study population are presented in Table 1. Ages ranged from 49 to 96 years, with a mean of 65.5. The population was mainly Caucasian with a minority (0.7%) of Aboriginal, Negroid, Oceanian, Asian, and Indian ethnicity. The mean VDD was 1.51 mm and the mean VCDR was 0.43. The population distributions of VDD and VCDR are shown in Figure 2. No statistically significant association was found between VDD and any of the studied covariates. Therefore, no additional correction other than standardization was made to the VDD data before commingling analysis. Skewness and kurtosis of the standardized VDD distribution were 0.191 and 0.152, respectively. VCDR was significantly associated with age (multivariate regression coefficient [B] = 0.001, P < 0.001), VDD (B = 0.003, P < 0.001) and IOP (B = 0.004, P < 0.001). The standardized residuals of this multivariate regression model were used for further analysis. The distribution of the standardized and adjusted VCDR data had a skewness of −0.068 and a kurtosis of −0.097.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number</td>
<td>3273</td>
</tr>
<tr>
<td>Age (y), mean ± SD</td>
<td>65.5 ± 9.4</td>
</tr>
<tr>
<td>Age 49–59 y, n (%)</td>
<td>964 (29.5)</td>
</tr>
<tr>
<td>Age 60–69 y, n (%)</td>
<td>1227 (37.5)</td>
</tr>
<tr>
<td>Age 70–79 y, n (%)</td>
<td>832 (25.4)</td>
</tr>
<tr>
<td>Age 80+, n (%)</td>
<td>250 (7.6)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>1438 (43.9)</td>
</tr>
<tr>
<td>White, n (%)</td>
<td>3248 (99.3)</td>
</tr>
<tr>
<td>VDD (mm), mean ± SD</td>
<td>1.51 ± 0.17</td>
</tr>
<tr>
<td>VCDR, mean ± SD</td>
<td>0.43 ± 0.14</td>
</tr>
<tr>
<td>Intraocular pressure (mm Hg), mean ± SD</td>
<td>16.0 ± 2.7</td>
</tr>
</tbody>
</table>

FIGURE 2. (A) Distribution of vertical disc diameter of random eye. (B) Distribution of VCDR of random eye.
Commingling Analysis of VDD

The results of the commingling analysis of VDD are presented in Table 2. Under the hypothesis of one distribution, significant skewness was removed by the power transformation (\(\chi^2 = 9.16, P = 0.027\) after Bonferroni correction). This is reflected by the findings that the skewness of the untransformed data was 0.191, whereas after the power transformation (with \(R\) fixed as 11.0 and \(P\) optimized by SKUMIX/SKUDRIVER as 0.35) the skewness was 0.002. The power transform did not significantly improve the fit of the data when two or three distributions were specified. For the untransformed data, the two-distribution model fitted the data significantly better than the one-distribution model (\(\chi^2 = 10.92, P = 0.047\) after Bonferroni correction), but the three-distribution model did not fit better than the two-distribution model. Considering the transformed models only, neither dataset provided evidence of commingling. Allowing the inbreeding coefficient (fixed) 0. These parameters gave rise to a considerable degree of model-selection uncertainty.

\[\chi^2 (df)\] Compared with
\[\chi^2 (df)\] Compared with
\[\chi^2 (df)\] Compared with

Commingling Analysis of VCDR

The results of the commingling analysis of VCDR, after adjustment for the effects of age, VDD, and IOP, are presented in Table 3. When the models were compared by means of maximum-likelihood ratio tests and the probabilities corrected for multiple comparisons by using the Bonferroni method, the data provided no significant evidence of skewness or commingling. However, when considering the AIC, the best fitting model was the three-distribution transformed model. This model had a 0.25 probability of fitting best, but was closely followed by the three-distribution untransformed model with a 0.22 probability, resulting in an evidence ratio of 1.14 for the relative likelihood of the transformed versus the untransformed three-distribution model. Neither model was improved by allowing the inbreeding coefficient to vary. The parameters of the three-distribution transformed model were: residual variance 0.44, homozygote mean = 0.07, dominance 0.46, displacement = 2.73, allele frequency 0.23, power transform variable = 0.57, power transform variable \(R\) (fixed) 11.0, inbreeding coefficient (fixed) 0. These parameters gave rise to the distributions shown in Figure 3. When the back-transformed, unstandardized regression residuals were considered, the middle distribution (which represents individuals carrying 1 copy of the rare allele) had a mean of −0.15, and the leftmost distribution (which represents individuals carrying two copies of the rare allele) had a mean of −0.29. As the total variance

### Table 2. Commingling Analysis of VDD

<table>
<thead>
<tr>
<th>Model</th>
<th>−2 × Log Likelihood + Constant</th>
<th>(\chi^2 (df)) Compared with Untransformed*</th>
<th>(\chi^2 (df)) Compared with 1 Distribution†</th>
<th>(\chi^2 (df)) Compared with 2 Distributions‡</th>
<th>Akaike Information Criterion§</th>
<th>Akaike Weight¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Distribution, untrans</td>
<td>4643.69</td>
<td>9.16 (1)†</td>
<td>10.92 (2)‡</td>
<td>0.25</td>
<td>4640.77</td>
<td>0.26</td>
</tr>
<tr>
<td>1 Distribution, trans</td>
<td>4643.52</td>
<td>9.16 (1)†</td>
<td>10.92 (2)‡</td>
<td>0.25</td>
<td>4640.77</td>
<td>0.26</td>
</tr>
<tr>
<td>2 Distribution, untrans</td>
<td>4641.11</td>
<td>1.66 (1)</td>
<td>3.42 (2)</td>
<td>0.02</td>
<td>4641.11</td>
<td>0.22</td>
</tr>
<tr>
<td>2 Distribution, trans</td>
<td>4641.87</td>
<td>11.82 (3)</td>
<td>0.90 (1)</td>
<td>0.02</td>
<td>4641.87</td>
<td>0.15</td>
</tr>
<tr>
<td>3 Distribution, untrans</td>
<td>4641.08</td>
<td>0.79 (1)</td>
<td>3.44 (3)</td>
<td>0.02</td>
<td>4643.08</td>
<td>0.08</td>
</tr>
<tr>
<td>3 Distribution, trans</td>
<td>4641.08</td>
<td>0.79 (1)</td>
<td>3.44 (3)</td>
<td>0.02</td>
<td>4643.08</td>
<td>0.08</td>
</tr>
</tbody>
</table>

* Difference between −2 log likelihood of the given model and the untransformed model for the same number of distributions.
† Difference between −2 log likelihood of the given model and the corresponding (i.e. transformed or untransformed) model for 1 distribution.
‡ Difference between −2 log likelihood of the given model and the corresponding (i.e. transformed or untransformed) model for 2 distributions.
§ −2 log likelihood + twice the number of free parameters.
¶ Relative likelihood of the model.

### Table 3. Commingling Analysis of VCDR

<table>
<thead>
<tr>
<th>Model</th>
<th>−2 × Log Likelihood + Constant</th>
<th>(\chi^2 (df)) Compared with Untransformed*</th>
<th>(\chi^2 (df)) Compared with 1 Distribution†</th>
<th>(\chi^2 (df)) Compared with 2 Distributions‡</th>
<th>Akaike Information Criterion§</th>
<th>Akaike Weight¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 distribution, untrans</td>
<td>4635.09</td>
<td>1.18 (1)</td>
<td>5.06 (2)</td>
<td>2.48 (1)</td>
<td>4638.03</td>
<td>0.17</td>
</tr>
<tr>
<td>1 distribution, trans</td>
<td>4635.91</td>
<td>1.18 (1)</td>
<td>5.06 (2)</td>
<td>2.48 (1)</td>
<td>4638.03</td>
<td>0.17</td>
</tr>
<tr>
<td>2 distribution, untrans</td>
<td>4630.03</td>
<td>2.19 (1)</td>
<td>6.07 (2)</td>
<td>2.48 (1)</td>
<td>4637.84</td>
<td>0.19</td>
</tr>
<tr>
<td>2 distribution, trans</td>
<td>4632.84</td>
<td>2.19 (1)</td>
<td>6.07 (2)</td>
<td>2.48 (1)</td>
<td>4637.84</td>
<td>0.19</td>
</tr>
<tr>
<td>3 distribution, untrans</td>
<td>4627.55</td>
<td>7.54 (3)</td>
<td>2.48 (1)</td>
<td>2.48 (1)</td>
<td>4637.55</td>
<td>0.22</td>
</tr>
<tr>
<td>3 distribution, trans</td>
<td>4625.35</td>
<td>8.58 (3)</td>
<td>2.51 (1)</td>
<td>2.51 (1)</td>
<td>4637.33</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Commingling analysis was performed after adjustment for the effects of age, vertical disc diameter, and intraocular pressure. Untrans, untransformed; trans, transformed.

* Difference between −2 log likelihood of the given model and the untransformed model for the same number of distributions.
† Difference between −2 log likelihood of the given model and the corresponding (i.e. transformed or untransformed) model for 1 distribution.
‡ Difference between −2 log likelihood of the given model and the corresponding (i.e. transformed or untransformed) model for 2 distributions.
§ −2 Log likelihood + twice the number of free parameters.
¶ Relative likelihood of the model.
analyses of VCDR resulted in a three-distribution model with
when patients with glaucoma were included, commingling
ecessary because we do not know whether the same processes
are not respected, and environmental factors of major effect are un-
this gene would reduce VCDR by 0.15 in heterozygotes (36% of
accounting for 58% of the variance in VCDR. The rare allele of
mixing of three distributions and would be compatible with
adjustment for the effects of age, VDD, and IOP, consisted of a
major gene effect. The most satisfactory model for VCDR, after
We sought to test the hypothesis of a major genetic determi-
ance due to the commingling was 0.61 and the locus-specific
was 1.05, the residual variance of 0.44 implied that the vari-
age of VCDR is biologically plausible and compatible
that accounts for 58% of the variance in VCDR. The rare allele of
in the population. This finding warrants some speculation
multifactorial transmission with ocular hypertension and in healthy individuals.34–37 How-
results for VDD therefore do not preclude a genetic determi-
important part of this additive genetic variance may be attributable to
The parameters of the best-fitting model indicated that the
rare allele of the major locus would cause a significant and
of VDD did not change after the glau-
cases were included.
linkage and asso-
additive effects of all
numbers provided evidence of segregation of a single locus but not of commingling. Our
results for VDD therefore do not preclude a genetic determi-
that the latter assessed the additive effects of all
involved genes. A collection of several loci with small effects
rather than a single major gene determining VDD may lead to
heritability estimates without evidence of commin-
notable.32 When both commingling and segregation analyses were
applied to simulated pedigree data in which a major locus was
segregating, more than 20% of the samples provided evidence of
of the population. This study was an actual risk factor or rather an early sign of
POAG, and consequently, whether the smaller VCDR associ-
identifying the gene and exploring its function
major genetic effect. The results for VDD therefore do not preclude a genetic determi-
sample size of approximately 450 and 150 sib pairs to have an
80% power to be detected by genome-wide linkage and associa-
tion methods, respectively.58,59 If a dichotomous trait-
based association analysis were to be considered, it would be
desirable to compare individuals having at least one copy of the
rare allele with individuals having no copies. The former group
would consist of individuals with VCDRs smaller than the
lower extreme of the rightmost distribution, say those with
VCDR values less than three residual standard deviations from

**DISCUSSION**

We sought to test the hypothesis of a major genetic determi-
nent of VDD and VCDR by analyzing the distribution of these
traits in a large Australian population. Commingling analysis of
VDD did not provide statistically significant evidence of a
major gene effect. The most satisfactory model for VCDR, after
adjustment for the effects of age, VDD, and IOP, consisted of a
mixture of three distributions and would be compatible with
the presence of a major gene with minor allele frequency 0.23
accounting for 58% of the variance in VCDR. The rare allele of
this gene would reduce VCDR by 0.15 in heterozygotes (36% of
the population) and by 0.29 in homozygotes (6% of the popu-
lation). The design of our study had two important limitations. First,
commingling analysis can provide evidence of a mixture of
distributions but cannot reveal the origin of the mixing com-
ponent. Evidence of commingling therefore does not necessar-
ily imply evidence of a major genetic effect, as environmental
sources of commingling cannot be ruled out. However, as a
genetic origin of VCDR is biologically plausible and compatible
with previous literature,12–14 Hardy-Weinberg proportions are
respected, and environmental factors of major effect are un-
known, it is likely that a gene of major effect explains this
commingling. Second, the exclusion of patients with glaucoma
is a possible source of selection bias. The exclusion was nec-
 essary because we do not know whether the same processes
are responsible for VCDR in healthy and glaucomatous eyes.
When patients with glaucoma were included, commingling
analysis of VCDR resulted in a three-distribution model with
dominance −0.59, displacement 2.05, and allele frequency
0.17. The rightmost distribution in this model (n = 93; SD = 0.75; mean = 2.05, corresponding to 0.27 unstandardized
residuals) was very similar to the distribution of the included
 glaucoma population (n = 83; SD = 1.05; mean = 2.04,
corresponding to 0.27 unstandardized residuals). This result
may indicate that patients with glaucoma form a separate
distribution and that the SKUMIX program is able to correctly
disentangle this admixture. However, a major genetic origin of
the commingling in this heterogeneous population could be
disputed. The results for VDD did not change after the glau-
coma population was included.

The lack of distributional effects in VDD in our study ap-
pears to disagree with previous work, in which heritability
estimates of VDD or disc area ranged from 0.52 to 0.73.11–13
Our result may be explained by the conservative design of the
SKUMIX program, which implements the commingling analy-
ysis. This design, which “had to guard against claiming separate
distributions where none exist”15 has been tested experimen-
tally.32 When both commingling and segregation analyses were
applied to simulated pedigree data in which a major locus was
segregating, more than 20% of the samples provided evidence of
segregation of a single locus but not of commingling. Our
results for VDD therefore do not preclude a genetic determi-
nant of major effect. This finding is also suggested by the
Akaike weights, which show a considerable model selection
uncertainty and provide some support for a two-distribution
model (fully dominant or recessive gene) as well. Another
possible explanation of the discrepancy with previous herita-
bility studies is that the latter assessed the additive effects of all
involved genes. A collection of several loci with small effects
rather than a single major gene determining VDD may lead to
high heritability estimates without evidence of commin-
ning.27,35

Additive genetic effects have been reported to account for
48% to 65% of the total variance in VCDR.12–14 Our estimate of
58% for its locus-specific heritability suggested that an impor-
tant part of this additive genetic variance may be attributable to
the effect of a single locus. Moreover, our study provided a
model elucidating the allele frequencies, dominance, and
displacement associated with this locus.

The parameters of the best-fitting model indicated that the
rare allele of the major locus would cause a significant and
clinically detectable reduction in VCDR in a substantial propor-
tion of the population. This finding warrants some specula-
tion on the clinical relevance of this potential locus. VCDR has been
reported to predict the development of POAG in individuals
with ocular hypertension and in healthy individuals.54–57 How-
ever, one might question whether a large VCDR in these
studies was an actual risk factor or rather an early sign of
POAG, and consequently, whether the smaller VCDR associ-
ated with the major locus in our study would actually reduce
POAG risk. Identifying the gene and exploring its function
could shed light on this issue.

Our results provide some guidance for the planning of
future gene-finding studies in this population. The commingled
model with the high locus-specific heritability of VCDR
strongly supports a quantitative trait-based approach. A gene
that accounts for 58% of the trait variance would require
sample sizes of approximately 450 and 150 sib pairs to have an
80% power to be detected by genome-wide linkage and associa-
tion methods, respectively.58,59 If a dichotomous trait-
based association analysis were to be considered, it would be
desirable to compare individuals having at least one copy of the
rare allele with individuals having no copies. The former group
would consist of individuals with VCDRs smaller than the
lower extreme of the rightmost distribution, say those with
VCDR values less than three residual standard deviations from

![Figure 3. VCDR: transformed three-distribution model. This figure shows the model which, after commingling analysis, fitted the population VCDR data best. It consists of three normal distributions, each containing n subjects with a mean x0 and common standard deviation b. The unstandardized values corresponding to x0 are given in the bottom x-axis.](image-url)
the mean of this distribution; that is, standardized VCDR regression residuals of less than $-0.07 - 3 \cdot 0.66 = -2.05$. This translates to VCDRs that are at least 0.26 smaller than would be expected based on age, VDD, and IOP. The second group would be those with VCDRs greater than the upper extreme of the middle distribution; that is, those with standardized VCDR regression residuals greater than $-1.32 + 3 \cdot 0.66 = 0.66$, corresponding to VCDR values of at least 0.08 greater than predicted from the covariates.

In conclusion, commingling analysis in this large, Australian population provided evidence of a mixture of distributions in VCDR. The result was consistent with the presence of a major gene accounting for 58% of the total variance in VCDR. Although a high heritability of VCDR has been reported, our study is the first to suggest that a major gene may be responsible for this trait. This finding strongly supports further efforts to identify the genetic variants responsible for VCDR, which is an important feature of the POAG phenotype.

References


