Linkage to 10q22 for Maximum Intraocular Pressure and 1p32 for Maximum Cup-to-Disc Ratio in an Extended Primary Open-Angle Glaucoma Pedigree

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PURPOSE. The purpose of this study was to identify genetic contributions to primary open-angle glaucoma (POAG) through investigations of two quantitative components of the POAG phenotype.

METHODS. Genome-wide multipoint variance–components linkage analyses of maximum recorded intraocular pressure (IOP) and maximum vertical cup-to-disc ratio were conducted on data from a single, large Australian POAG pedigree that has been found to segregate the myocilin Q368X mutation in some individuals.

RESULTS. Multipoint linkage analysis of maximum recorded IOP produced a peak LOD score of 3.3 (P = 0.00015) near marker D10S537 on 10q22, whereas the maximum cup-to-disc ratio produced a peak LOD score of 2.3 (P = 0.00056) near markers D1S197 to D1S220 on 1p32. Inclusion of the myocilin Q368X mutation as a covariate provided evidence of an interaction between this mutation and the IOP and cup-to-disc ratio loci.

CONCLUSIONS. Significant linkage has been identified for maximum IOP and suggestive linkage for vertical cup-to-disc ratio. Identification of genes contributing to the variance of these traits will enhance understanding of the pathophysiology of POAG as a whole. (Invest Ophthalmol Vis Sci. 2005;46:3723–3729) DOI:10.1167/iovs.05-0312

G}lauc}oma 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,5 whereas under-reporting of family history suggests that the genetic component of POAG may be even greater than is generally acknowledged.6

The clinical diagnosis of glaucoma is based on a combination of several main features, including specific changes to the optic nerve head constituting glaucomatous optic neuropathy, characteristic visual field loss with a slow and often asymptomatic progression, and, in most cases, increased intraocular pressure (IOP). The complexity of the phenotypic definition of POAG6,7 has contributed to the difficulties in identifying genes involved in this disease.

Two genes have been identified for adult-onset POAG8–9, however, mutations in these genes account for only a fraction of POAG cases. Myocilin (MYOC) on 1q24.3 has been shown to account for approximately 3% of adult-onset POAG.9–12 Optineurin (OPTN) on 10p15-p14 was shown by Rezaie et al.10 to be involved in up to 17% of low-tension glaucoma pedigrees, although a recent study indicated a low prevalence of OPTN mutations (<0.1%) in unselected cases of both POAG and NTG.11,12 The WD40-repeat 36 gene (WDR36) was recently identified at the GLC1G locus on 5q22.1,14 although the impact of this gene on the wider POAG population is yet to be determined. In addition to these three genes, four other loci for POAG have been mapped: GLC1B,15 GLC1C,16 GLC1D,17 and GLC1E.18 The results of three genome-wide scans suggest that several additional chromosomal regions may also be involved in susceptibility to POAG19–21; however, only GLC1B22 and GLC1C23 have been replicated in published studies.

Given the limited success so far in identifying genes conferring susceptibility to glaucoma, one approach that may have greater success is quantitative trait linkage analysis of precursors of glaucoma, such as raised IOP and increased cupping of the optic nerve. Such traits may have simpler genetic architecture than diagnosis of glaucoma, making it easier to map causative loci.24–26 Furthermore, quantitative trait linkage analysis is inherently more powerful than dichotomous trait linkage analysis27,28 and is particularly powerful in large families.29,30

The heritabilities of IOP and vertical cup-to-disc ratio were estimated to be 0.36 and 0.48, respectively, in the Beaver Dam Eye Study, providing evidence for genetic determinants for these components.31 Commingling analysis of IOP and glaucoma by Viswanathan et al.32 suggested the existence of a major gene accounting for 18% of the variance of IOP in the Blue Mountains Eye Study population.32 Duggal et al.33 recently conducted a complex segregation and linkage analysis

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of IOP, identifying two potential regions of linkage on chromosomes 6 and 13.

The purpose of the present investigation was to identify regions of linkage that contribute to maximum IOP and maximum cup-to-disc ratio, using measures from an extended Australian pedigree. This family already has been analyzed for linkage to glaucoma. The discovery of genes contributing to the variance of maximum IOP and maximum cup-to-disc ratio is expected to provide significant insights into the pathophysiology of glaucoma.

METHODS

The Glaucoma Inheritance Study in Tasmania

This investigation was conducted as part of the Glaucoma Inheritance Study in Tasmania (GIST), a large population study of glaucoma-affected families in Tasmania, Australia. Ethics approval was obtained from the Human Research Ethics Committees of the Royal Children’s Hospital, the Royal Victorian Eye and Ear Hospital, the Royal Hobart Hospital, and the University of Tasmania, and the study was conducted in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants.

GTas02, the family of interest in this investigation, is one of the largest pedigrees identified as part of the GIST. It consists of more than 1350 members, and ancestry can be traced back to a founder couple six generations ago. The “core” pedigree containing the POAG cases consists of 246 individuals when deceased linking members are included. One hundred thirty-nine family members consented to clinical examination and blood collection for genotyping and mutation analysis. The structure, clinical diagnosis, and MYOC mutation status of this family have been published.

Clinical Examination

Clinical examination and diagnosis of patients involved in the GIST are documented elsewhere. In brief, POAG was clinically defined as an optic neuropathy that had at least two of the following features: (1) optic nerve head excavation with thinning of the neuroretinal rim, often with Drance-type nerve fiber layer hemorrhages, notching, pit-ting, significant focal loss or general loss of the retinal nerve fiber layer (generally measured by an enlarged vertical cup-to-disc ratio ≥ 0.7); (2) elevated IOP above a population-based normal range or above the average of the unaffected individuals within a pedigree (generally IOP ≥ 21 mm Hg or two standard deviations from the population mean); and (3) visual field defects consistent with the disc changes and with common descriptions of glaucomatous field loss. Glaucoma cases secondary to trauma or anterior segment dysgenesis were excluded. Of the 139 individuals available for examination, 24 had a diagnosis of POAG. The details of the clinical diagnoses for this family have been extensively reported.

IOP was measured with a calibrated Goldmann applanation tonometer. Multiple IOP measures were available for each individual; hence, maximum IOP was selected, to reduce any bias introduced by using postmedication pressure values. No corrections were made for corneal thickness, because this information was not routinely collected at the time of patient ascertainment. Optic disc appearance was classified by two clinicians at the time of examination, with a slit lamp biomicroscope after pupil dilation. In addition, optic disc stereo photographs (Nidek, Gamagori, Japan) were obtained for future reference in all cases. When there was a discrepancy between the two examiners, the stereo disc photographs were independently assessed by a glaucoma specialist. The highest vertical cup-to-disc ratio in either eye at any clinical examination was used as the trait measure.

Quantitative measures collected as part of the clinical examination of patients with POAG and their relatives included maximum recorded intraocular pressure (IOP) without medication and maximum vertical cup-to-disc ratio from the highest-scoring eye.

Myocilin mutation detection in this family has been reported. Of the 139 individuals from GTas02 screened for mutations in the MYOC gene, 19 were known to carry the Q368X mutation.

FIGURE 1. Genome-wide multipoint variance components linkage results for maximum recorded IOP.
Genotyping
A 10-cM genome-wide scan was conducted with 401 microsatellite markers from fluorescence marker sets (vers. 1 and 2; Applied Biosystems, Inc. [ABI], Foster City, CA), run on sequencers (model 377; ABI) and analyzed (GeneScan and GenoTyper software; ABI).

Ascertainment Correction
The population ascertainment correction data set was taken from the Melbourne Visual Impairment Project (MVIP) and included 3905 individuals from the general population with data on age, sex, maximum recorded IOP, and maximum cup-to-disc ratio. The MVIP clinical data were collected by the same methods as were used in the GIST. A 10-cM genome-wide scan was conducted with 401 microsatellite markers from fluorescence marker sets (vers. 1 and 2; Applied Biosystems, Inc. [ABI], Foster City, CA), run on sequencers (model 377; ABI) and analyzed (GeneScan and GenoTyper software; ABI).

Variance Components Analysis
Heritability for each trait was estimated with genetic variance component modeling as implemented in SOLAR (ver. 2.1.1). The covariates were selected among age, sex, age–sex interaction; however, age–sex interaction was not significant in any dataset and was thus removed. Variance component linkage analysis was performed to detect and localize quantitative trait loci (QTLs) influencing variation in maximum IOP and maximum cup-to-disc ratio, by using SOLAR.

The variance-component linkage method is based on specifying the expected genetic covariances between arbitrary relatives as a function of IBD relationships at a given marker locus. The method involves partitioning the total trait phenotypic variance ($\sigma^2_P$) into components attributable to covariate effects, effects of a specific QTL (QTL ($\sigma^2_{Q}$), and residual additive genetic effects ($\sigma^2_{A}$)). The test for linkage compares the likelihood of this model with the likelihood of a null model (no linkage), where the QTL effect size ($\sigma^2$) is fixed to be zero. The difference between the two $\log_{10}$ likelihoods produces a LOD score that can be interpreted in a fashion similar to that of the classic LOD scores of parametric linkage analysis. The variance component quantitative genetic approach enables penetrance model-free multipoint linkage analysis of complex quantitative traits in pedigrees of arbitrary size and complexity (25-28, 45-47) and has been used successfully to localize QTLs that influence many important disease-related traits, including risk of alcoholism, serum leptin levels, and resting heart rate.

Expected LOD scores and empirical locus-specific probabilities were determined with the “lodadj” command of SOLAR. A total of 100,000 replicates were simulated to build up the distribution of LOD scores expected under the null hypothesis of no linkage, using a fully informative, unlinked marker. The observed LOD scores were then regressed on those expected for a multivariate normal trait, with the inverse slope of the regression line providing the LOD correction constant.

Expected LOD scores were multiplied by the correction constant only if the constant was <1.

**Figure 2.** Multipoint variance-components linkage results for maximum recorded IOP for chromosome 10 (solid line) and linkage signal after the inclusion of Q368X status as a covariate (dashed line). Covariates are indicated on the plots. The location of the optineurin (OPTN) gene is indicated.
Maximum recorded IOP had a heritability of 0.55 for family GTsas02. The covariates sex and age were statistically controlled for and included in the analysis, although in the ascertainment correction population, neither showed effects that were substantial ($P = 0.073$ for sex, $P = 0.55$ for age), and accounted for only 0.1% of the variance. The mean value for maximum recorded IOP in family GTsas02 (17.9 ± 4.2 mm Hg [SD]) was significantly higher ($P < 0.0001$) than the mean IOP in the general population (15.2 ± 3.3 mm Hg). The empirical LOD correction constant was greater than 1; hence, no adjustment was applied to the linkage results. The highest LOD score for IOP in family Gtsas02 was 3.3 (locus-specific $P = 0.00015$) near marker D10S537 (Fig. 1), with the LOD-1 interval spanning approximately 20 cM on 10q22 (Fig. 2). The genome-wide probability for this result was 0.0165.

Maximum cup-to-disc ratio had a heritability of 0.39 for family Gtsas02. The effect of the covariates sex ($P = 3.1 \times 10^{-5}$) and age ($P = 1.6 \times 10^{-5}$) were highly significant in the ascertainment correction population, accounting for 1.4% of the variance; hence, both were statistically controlled for and included in the analysis of family Gtsas02. The mean ± SD cup-to-disc ratio in family Gtsas02 (0.47 ± 0.20) was not significantly different ($P > 0.05$) from the mean cup-to-disc ratio in the general population (0.43 ± 0.21). The empirical LOD correction constant was greater than 1; hence, no adjustment was applied. The highest LOD score for maximum cup-to-disc ratio in family Gtsas02 was 2.3 (locus-specific $P = 0.00056$) near markers D1S197 and D1S220 (Fig. 3), with the LOD-1 interval spanning approximately 20 cM on 1p32 (Fig. 4). The genome-wide probability for this LOD score was determined to be 0.208.

The MYOC Q368X mutation was present in 9 (37.5%) of 24 individuals in family Gtsas02 with diagnosed POAG and in 10 individuals without a diagnosis of POAG at examination. Whereas the mean age of individuals with the Q368X mutation in family Gtsas02 was not significantly different from those without the mutation, mean maximum IOP and mean maximum cup-to-disc ratio were significantly higher in Q368X mutation carriers, regardless of POAG affection status (Table 2). MYOC Q368X mutation status ($\beta_{MYOC}$) accounted for 16% of the genetic variance of the maximum cup-to-disc ratio (heritability reduced from 0.39 to 0.20). Addition of MYOC Q368X mutation status as a covariate in the analysis of the quantitative traits resulted in the peak LOD score for maximum recorded IOP (near marker D10S537 on 10q22) decreasing from 3.3 to 1.9 (Fig. 2), and the peak LOD score for maximum cup-to-disc ratio (near markers D1S197 and D1S220 on 1p32) was reduced from 2.3 to 0.9 (Fig. 4).

**DISCUSSION**

In this study, we investigated two individual disease components of POAG—maximum recorded IOP and maximum vertical cup-to-disc ratio—as quantitative traits using variance components linkage analysis. Genome scan analyses of these traits in an extended pedigree revealed one region of significant or suggestive linkage for each trait. Multipoint linkage analysis of maximum recorded IOP identified a peak LOD of 3.3 (locus-specific $P = 0.00015$) near marker D10S537 on 10q22, whereas analysis of the maximum cup-to-disc ratio produced a peak LOD score of 2.3 (locus-specific $P = 0.00056$) near markers D1S197 to D1S220 on 1p32.

The putative trait locus for maximum recorded IOP produced a significant peak LOD of 3.3 on 10q22. The genome-wide significance level of this result ($P = 0.0165$) strongly suggests that this region contains a gene that contributes to the variance of IOP. We did not see any overlap with the IOP linkage regions identified by Duggal et al. Although our region of interest for IOP on 10q22 has not been reported previously for IOP, linkage to this region has also been found for systemic hypertension in a Japanese population. The association between systemic blood-pressure (systolic or diastolic) and IOP has been well documented. It is possible
that systemic hypertension and IOP share a common QTL in this region on the long arm of chromosome 10. The region contains BMPR1A (MIM: 601299), a bone morphogenic protein receptor—interesting because Bmp4 has been implicated in increased IOP in mice.\(^\text{56}\) The peak region also contains RGR (MIM: 600342), an opsin-related gene associated with retinitis pigmentosa.\(^\text{57}\) The OPTN gene is located outside our 10p13 linkage peak, approximately 60 cM upstream.

Applanation tonometry measurements of IOP are known to be influenced by central corneal thickness (CCT).\(^\text{58,59}\) CCT has a positive and apparently linear correlation with IOP\(^\text{60}\) and is strongly genetically determined (Toh TY, et al. IOVS 2005;46: ARVO E-Abstract 1093). There has been a report of thick corneas segregating in a family with apparent ocular hypertension,\(^\text{61}\) which leads to the suggestion that perhaps the variation in IOP in family GTas02 compared with the population is due to CCT. However, there are many individuals in family GTas02 with moderate to advanced visual field loss, which would support the elevated IOP's being genuine rather than artifactual in nature. It is possible that measurement of CCT and subsequent adjustment of Goldmann applanation IOP readings for GTas02 may have the effect of strengthening the linkage at the IOP locus on 10q22 by providing a more accurate approximation of true IOP (as opposed to measured IOP). This approach is worthy of future investigation.

**FIGURE 4.** Multipoint variance components linkage results for maximum vertical cup-to-disc ratio for chromosome 1 (solid line), and linkage signal after the inclusion of Q368X status as a covariate (dashed line). Covariates are indicated on the plots. The location of the myocilin (MYOC) gene is indicated.

**TABLE 2.** Comparison between Myocilin Q368X Mutation Carriers and Mutation-Free Individuals from Family GTas02

<table>
<thead>
<tr>
<th>Trait</th>
<th>Q368X Mutation Carriers</th>
<th>Q368X Mutation Free</th>
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<tr>
<td></td>
<td>n</td>
<td>Mean</td>
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<tr>
<td>Maximum IOP</td>
<td>19</td>
<td>22.21</td>
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<tr>
<td>Maximum cup-to-disc ratio</td>
<td>19</td>
<td>0.64</td>
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\(P^*\) Obtained by age-adjusted measured genotype analysis accounting for nonindependence among relatives.
two quantitative traits investigated in this study. Because only 9 of the 19 Q368X mutation carriers in this family were clinically diagnosed with POAG, this mutation is unable to account for the presence of all cases of POAG in the entire family. Despite the absence of a linkage signal at MYOC, the Q368X mutation appeared to account for nearly half the genetic variance for the quantitative trait based on maximum cup-to-disc ratio, and nearly 20% of the genetic variance for maximum IOP. The differences between carrier and non-carrier-trait values (shown in Table 2) were significant, which may indicate epistasis or some other form of interaction between MYOC and the putative trait loci for IOP and cup-to-disc ratio. The reduction in peak LOD scores for both traits after the inclusion of the Q368X mutation as a covariate provides further evidence for the presence of an interaction. However, the sample size of family GTas02 does not provide sufficient power to determine the exact mechanism behind this interaction. Subsequent identification of the genes at these loci is anticipated to aid investigation into the nature of this interaction.

Baird et al. recently identified linkage of clinical POAG diagnosis to 3p21-p22 in this family, using MCMC-based linkage analysis. We did not find any evidence of overlap with this region using the traits maximum IOP and cup-to-disc ratio. There are likely to be many genes contributing to the complex POAG phenotype, and different analytical approaches will have variable power to detect certain loci. It is also possible that the gene at the 3p locus may contribute to a different POAG trait, such as progression from elevated IOP to optic nerve damage and subsequent clinical signs of visual field loss. Although we are unaware of any systemic biases in pedigree or family member ascertainment, genotyping methodology, or trait measurements, if any such biases exist they would impact both linkage studies and any subsequent analyses of this data set. Finally, as with any genome scan, one must also be mindful that some of these linkage results could represent chance events.

The loci identified in this study are believed to contribute to the variance of IOP and cup-to-disc ratio in the general population. However, since more extreme values are present in a POAG pedigree, when combined with population-based ascertainment correction, the approach used in this investigation is expected to provide greater power to map genes for these traits. Maximum IOP and maximum vertical cup-to-disc ratio may not be ideal measures, given the influence of pressure spikes and diurnal variation on IOP, for example, however other traits such as mean IOP are likely to be equally problematic considering the probable inclusion of postmedication levels. In addition, any measurement error in IOP and cup-to-disc ratio would also be present in the population data used for ascertainment correction.

In this investigation, we were able to identify regions of linkage contributing to maximum recorded IOP and maximum cup-to-disc ratio, quantitative clinical contributors to POAG diagnosis that are collected as a routine part of clinical practice. The discovery of the genes involved in these components of POAG at these loci is anticipated to provide significant insights into glaucoma pathophysiology as a whole.

Acknowledgments

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References


