GLC1F, A New Primary Open-angle Glaucoma Locus, Maps to 7q35-q36

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Background: A large family with adult-onset primary open-angle glaucoma (POAG) was identified.

Objective: To initiate a genome-wide scan to map the POAG locus in this family.

Methods: Blood samples or buccal swabs were obtained from 25 members of a large family with POAG after informed consent was obtained. Members and their spouses were evaluated clinically for POAG on the basis of intraocular pressures, cupping of discs, and visual fields. DNA samples were used for a genome-wide screen using microsatellite markers.

Results: Ten affected family members in 4 generations showed evidence of POAG including intraocular pressures of 22 mm Hg or more, and/or optic cup–disc ratios of 0.6 or more, and/or visual field defects consistent with glaucomatous damage. Primary open-angle glaucoma segregated as an autosomal dominant trait, with the disease locus mapping to 7q35-q36 between markers D7S2442 and D7S483 with a multipoint lod score of 4.06.

Conclusion: A sixth gene for POAG (GLC1F) has been mapped to 7q35-q36 in a family with at least 4 generations affected.

Clinical Relevance: The mapping of this locus further confirms that primary open-angle glaucoma is a heterogeneous group of diseases with at least 6 different loci resulting in a similar phenotype. The eventual ability to classify which major POAG gene an affected person carries could have ramifications for selecting the most effective treatment regimen for that person.


PRIMARY OPEN-ANGLE glaucoma (POAG) results in a loss of central and peripheral vision, usually in a specific pattern as optic nerve fibers are destroyed.1 Most persons with POAG have high intraocular pressures (IOPs), optic nerve cupping, and characteristic visual field defects; however, the underlying disease process of POAG has yet to be determined.2 Risk factors for POAG include family history, race, and myopia.3 There is some basis for regarding elevated IOP as a risk factor for glaucomatous optic neuropathy.4 Primary open-angle glaucoma is the third leading cause of blindness worldwide,2 and it has been estimated that it will affect 68 million persons by the year 2000.4 Many forms of POAG may be polygenic; however, at least 5 major genes for POAG have been localized.7-12 The POAG loci are named GLC1, and a letter is added to indicate each new locus. The first POAG locus to be described, GLC1A, results from mutations in trabecular meshwork–induced glucocorticoid response protein, also known as myocilin.13-15 GLC1B maps to chromosome 2 and is interesting because it has a higher incidence of low-tension glaucoma than in the general population of patients with POAG.12 We mapped GLC1C in a large family with adult-onset POAG with the typical findings of glaucoma, including high IOPs.14 GLC1D and GLC1E have been mapped to 8q23 and 10p,14,15 respectively.9,10 The identification of 6 major POAG loci during the last 5 years suggests that POAG eventually may be described by as many if not more loci than retinitis pigmentosa.

We report the mapping of GLC1F to a 5.3-centimorgan (cM) region on chromosome 7q35-q36 in a family with adult-onset POAG.

RESULTS

Glaucoma in this family affected at least 4 generations and was consistent with autosomal dominant inheritance (Figure 1). No generations were skipped, and male-to-male transmission was observed. Four of the 8 children in the second generation were reportedly given a diagnosis of
PATIENTS AND METHODS

PATIENTS

Blood samples were obtained from 21 family members and 2 spouses, and buccal swabs were obtained from 2 persons from this family after informed consent was obtained. One of us (J.R.S.) examined 17 of the family members, including the 2 spouses. The medical records were obtained from the remaining persons' ophthalmologists. The present study was approved by the institutional review board at the Oregon Health Sciences University, Portland (approval No. 3352).

Family members were examined by gonioscopy with a Zeiss 4-mirror lens (Carl Zeiss, Inc, Thornwood, NY) and graded according to the Becker Schaffer grading system, with grade 4 indicating that the iridocorneal angle is 40° or more. Criteria for the diagnosis of POAG included a glaucomatous visual field defect or IOPs of 22 mm Hg or more and a vertical optic cup–disc ratio of 0.6 or more. All persons were considered unaffected, if their IOP was less than 20 mm Hg and their vertical optic cup–disc ratio was 0.3 or less. Increased pigmentation in the trabecular meshwork was not observed in any of the affected persons. Thus, all affected persons had POAG with no evidence of pigment dispersion syndrome.

MICROSATELLITE MARKER TYPING

DNA was isolated from the blood and buccal swab samples as previously described or by using a purification kit (MasterAmp Genomic DNA or MasterAmp Buccal Swab purification kit, Epicentre Technologies, Madison, Wis). Microsatellite markers as reported by Gypay et al were purchased from Research Genetics, Huntsville, Ala. A genome-wide search was conducted using microsatellite markers as previously described.

LINKAGE ANALYSIS

We used the VITESSE computer package for 2-point and multipoint linkage analysis. Estimation of the genetic model parameters for the analyses is discussed in detail in Wirtz et al. Briefly, we assumed autosomal dominant inheritance of a rare gene (frequency, 0.0001) with age-dependent penetrance. The stepwise age correction we used was based on the distribution of the age at onset for the 10 affected persons in this family (Table 1). This distribution was comparable to that in the family with GLC1C described earlier. We used a maximum penetrance estimate of 0.75 based on these family data, as well as published estimates. Only definitely affected persons were considered affected. We also conducted an affected persons-only analysis, in which only persons with definite POAG were coded as affected, and all others were considered unknown for disease status. In both types of analysis, we specified a phenocopy rate of 2%, based on population prevalence estimates of POAG.

We analyzed 5 linked marker loci that span 5.3 cM on chromosome 7q. The allele frequencies, map order, and distances between the Genethon markers reported by Dib et al were used.

Some regions were slightly positive, but when additional markers were tested, these regions were excluded. After excluding large regions of the genome, we eventually identified linkage to D7S636 and, subsequently, to several adjacent microsatellite markers. Two-point lod scores are reported in Table 2 for these markers, for the age-corrected and the affected persons-only analysis. The maximum pairwise lod score was obtained with D7S2439 (4.01 at $\theta = 0.0$ for age-corrected; 2.96 at $\theta = 0.0$ for affected persons only). Results of the multipoint analysis with POAG and these markers are shown in Figure 1. A multipoint lod score of 4.06 (age-corrected) extends across the 3-cM region from D7S305 to D7S2439, as does the lod score of 3.02 for affected persons only. These lod scores approach the maximum possible lod scores under these 2 models.

Haplotype data are shown in Figure 1. Critical crossovers occurred in 2 affected persons: 4048 shows a D7S2442-D7S505 crossover; 4037 shows a D7S2439-D7S483 crossover. Patients 4091 and 4088 inherited the disease haplotype from their father. However, they are both younger than 30 years and, thus, are probably too young to show signs of the disease. These data indicate that a gene for adult-onset POAG is located within a 5.3-cM region on the distal long arm of chromosome 7. This region is just proximal to the pigment dispersion syndrome mapped to 7q33-q36.
Table 1. Clinical Findings in Family Members With Primary Open-angle Glaucoma*

<table>
<thead>
<tr>
<th>Pedigree No.</th>
<th>Gonioscopy Stage</th>
<th>Age at Diagnosis, y</th>
<th>Optic Cup−Disc Ratio Before Medication</th>
<th>Visual Field</th>
<th>Ocular Medications†</th>
<th>LTP</th>
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<tbody>
<tr>
<td>4030</td>
<td>IV</td>
<td>40</td>
<td>0.7 0.8</td>
<td>32 32 19 19</td>
<td>Nasal step defect OD 2</td>
<td>None</td>
</tr>
<tr>
<td>4031</td>
<td>IV</td>
<td>65</td>
<td>0.8 0.8</td>
<td>36 36 28 28</td>
<td>Unreliable 1</td>
<td>OU</td>
</tr>
<tr>
<td>4037</td>
<td>IV</td>
<td>46</td>
<td>0.6 0.9</td>
<td>22 30 25 32</td>
<td>Superior arcuate scotoma OS 2 6</td>
<td>OS</td>
</tr>
<tr>
<td>4048</td>
<td>IV</td>
<td>44</td>
<td>0.6 0.3</td>
<td>22 22 NA NA</td>
<td>Unreliable None None None</td>
<td></td>
</tr>
<tr>
<td>4050</td>
<td>IV</td>
<td>69</td>
<td>0.7 0.7</td>
<td>25 22 NA NA</td>
<td>Normal None None</td>
<td></td>
</tr>
<tr>
<td>4053</td>
<td>IV</td>
<td>47</td>
<td>0.9 0.8</td>
<td>27 38 16 37</td>
<td>Superior arcuate scotoma OS; nasal step defect OS</td>
<td>1 5 OS</td>
</tr>
<tr>
<td>4080</td>
<td>IV</td>
<td>60s</td>
<td>0.7 0.6</td>
<td>NA NA 20 14</td>
<td>NA 1, 4, 6 OU</td>
<td></td>
</tr>
<tr>
<td>4087</td>
<td>IV</td>
<td>70</td>
<td>0.8 0.6</td>
<td>28 22 19 18</td>
<td>Constricted bilaterally 1 None</td>
<td></td>
</tr>
<tr>
<td>4087</td>
<td>IV</td>
<td>25</td>
<td>0.6 0.5</td>
<td>28 23 33 23</td>
<td>Normal 1 None</td>
<td></td>
</tr>
<tr>
<td>4080</td>
<td>IV</td>
<td>68</td>
<td>0.6 0.6</td>
<td>NA NA 20 20</td>
<td>Superior arcuate scotoma OS 3 None</td>
<td></td>
</tr>
</tbody>
</table>

*LTP indicates laser trabeculoplasty; NA, not available.
†Key to medications: 1, timolol maleate (Timoptic); 2, levobunolol hydrochloride (Betagan); 3, betaxolol hydrochloride (Betopic); 4, dorzolamide hydrochloride (Trusopt); 5, latanoprost (Xalatan); and 6, dipivefrin hydrochloride (Propine).

Figure 1. Pedigree of family with primary open-angle glaucoma (POAG) and haplotypes of 7q markers. Closed symbols denote patients with POAG; open symbols denote unaffected persons. Genotypes are listed in the order given by the map in the box at the lower right. Samples were available for all persons for whom a haplotype is drawn. The haplotype of the disease chromosome is boxed. The proband is indicated by the arrow.
While the specific mechanism by which each of these gene defects results in glaucoma has yet to be identified, general hypotheses can be made. Trabecular meshwork–induced glucocorticoid response protein was originally identified based on its response to glucocorticoids and oxidative stress. Forkhead transcription factor, RIEG, and LIM-homeodomain gene are transcription factors that are important in eye development. CYP1B1 is a member of a superfamily of hemoproteins that are involved in the oxidative metabolism of drugs and also respond to oxidative stress. The caveat to this line of reasoning is that congenital glaucoma, iridocorneal mesodermal dysgenesis, Axenfeld anomaly, iris hypoplasia, and nail-patella syndrome are considered developmental defects. Primary open-angle glaucoma is not an obvious developmental problem. However, the finding that adult-onset glaucoma results from mutations in the same genes that cause developmental defects, such as juvenile glaucoma and nail-patella syndrome, suggests a relationship may exist. Thus, transcription factors and genes that respond to oxidative stress are potential candidate genes based on the aforementioned hypothesis.

Fifty-seven expressed sequence tags have been mapped to the 5.3-cM region between D7S2442 and D7S483. Of the known genes in the region, only 2 are involved in transcription or oxidative stress. C2H2-150 is a KRAB domain–containing C2H2-type zinc-finger protein. C2H2-type zinc-finger proteins are transcription factors, and the KRAB domain may repress gene transcription. Several C2H2-type zinc-finger proteins are involved in developmental processes and tumorigenesis. Thus, C2H2-150 is a GLC1F candidate gene.

Nitric oxide synthase also maps to this region. Nitric oxide synthase is a glaucoma gene based on the aforementioned criteria and because it is involved in vasodilation and nitric oxide metabolism. Nitric oxide synthase is an oxidative stress protein and a cytochrome P-450-type hemoprotein. Interestingly, an association of a polymorphism in the 5' region of nitric oxide synthase in patients with familial POAG has been

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**Figure 2.** Optic disc photographs from patient 4048 show asymmetry between the left and right optic nerves.

**Figure 3.** Multipoint analysis with age correction in the full family shows a maximum lod score of 4.06. Recombination events occurring in patients 4048 and 4037 define a 5.3-cM region between D7S2442 and D7S483. Squares indicate affected persons only; circles, age-corrected.

**Table 2. Results of Pairwise Linkage Analyses of Chromosome 7 in Family With Primary Open-angle Glaucoma**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Lod Score at ( u = 0.00 )</th>
<th>Lod Score at ( u = 0.01 )</th>
<th>Lod Score at ( u = 0.05 )</th>
<th>Lod Score at ( u = 0.10 )</th>
<th>Lod Score at ( u = 0.20 )</th>
<th>Lod Score at ( u = 0.30 )</th>
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</thead>
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<tr>
<td>D7S2442</td>
<td>Age-corrected: -6.01, -3.55, -1.83, -1.05, -0.39, -0.13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Affected persons only: 1.24, 0.43, 0.94, 1.01, 0.83, 0.54</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>D7S505</td>
<td>Age-corrected: 2.12, 2.08, 1.92, 1.72, 1.32, 0.89</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Affected persons only: 1.37, 1.34, 1.24, 1.10, 0.84, 0.56</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D7S642</td>
<td>Age-corrected: 2.66, 2.60, 2.37, 2.07, 1.47, 0.87</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Affected persons only: 1.69, 1.65, 1.49, 1.30, 0.90, 0.51</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D7S2439</td>
<td>Age-corrected: 4.01, 3.93, 3.64, 3.27, 2.40, 1.59</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Affected persons only: 2.96, 2.91, 2.69, 2.41, 1.81, 1.16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D7S483</td>
<td>Age-corrected: 2.76, 2.70, 2.48, 2.20, 1.62, 1.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Affected persons only: 1.95, 1.91, 1.75, 1.54, 1.11, 0.67</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
reported. Thus, nitric oxide synthase and C2H2-150 are glaucoma candidate genes.

The finding of 6 POAG loci during the last 5 years indicates that POAG is a heterogeneous disease and may have a high correlation with POAG. In this article, we describe one of the families with high-IOP adult-onset POAG to be described with more than 4 generations involving at least 12 affected persons, 10 living and 2 now deceased. The phenotype of this family is fairly typical one with high IOPs; thus, there is no phenotypic difference that would help clinicians determine people at risk for GLC1F. To our knowledge, this is the first report of mapping of GLC1F to a 5.3-CM region on the long arm of chromosome 7.

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REFERENCES