A New Locus (GLC1H) for Adult-Onset Primary Open-angle Glaucoma Maps to the 2p15-p16 Region

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Objective: To map new genetic loci for adult-onset primary open-angle glaucoma (POAG) by using families previously unlinked to GLC1A-GLC1F.

Methods: Initial genome scan and subsequent saturation mapping confirmed linkage to a locus on chromosome 2p15-p16. Forty-nine DNA samples from a single family with POAG with 113 individuals were used in this study. The 10 affected members of this family had an average age at onset of 64 years, moderate to high intraocular pressure, glaucomatous visual field loss, and cup-disc ratios of 0.6 to 0.9.

Results: Haplotype construction in 9 available affected subjects established a single inherited chromosome from D2S123 to D2S2165, within an 11-megabase (Mb) region. Further analysis revealed no recombination with 8 consecutive DNA markers (D2S1364-D2S2332) and provided a maximum logarithm of the odds (LOD) score of 2.97 for D2S370. Six additional families also showed consistent linkage and 1 affected recombination may further confine this locus to an 8.3-Mb region (D2S2352-D2S2165). Combined analysis of 7 families provided a maximum LOD score of 9.30 with D2S2320.

Conclusions: A new genetic locus (GLC1H) for adult-onset POAG maps to the 2p15-p16 region.

Clinical Relevance: Mapping of the GLC1H locus and eventual identification of its defective gene will help to develop diagnostic tools and effective treatments for this condition.


GLAUCOMA IS A HETEROGENEOUS GROUP OF OPTIC NEUROPATHIES AND THE SECOND FOREMOST CAUSE OF BLINDNESS WORLDWIDE.1 This condition causes irreversible visual damage, yet early diagnosis and proper treatment may control disease progression. The most prevalent type, primary open-angle glaucoma (POAG), typically manifests with open angles and normal gonioscopy results, characteristic visual field loss, optic nerve damage, frequently elevated intraocular pressure (IOP), and absence of secondary causes of glaucoma.² Apoptosis of retinal ganglion nerve fibers usually leads to gradual characteristic loss of midperipheral vision and excavation of the optic disc in POAG.

The POAG mode of inheritance is variable and various parametric and nonparametric methods of linkage analysis have led to the mapping of 11 genetic loci (GLC1A-GLC1K).³-¹² The GLC1A gene Myocilin is responsible for about 36% of juvenile-onset¹³ and 2% to 4% of adult-onset POAG cases.¹⁴ The GLC1E gene optineurin is responsible for familial normal-tension glaucoma¹⁵ and more than 13 mutations identified so far. The GLC1G gene WDR36 originally showed mutations in 5% to 7% of POAG cases¹⁶ but other studies reported mutations in 10% to 17% of cases.¹⁶

To identify new chromosomal locations, we used a group of families with adult-onset glaucoma that previously were unlinked to GLC1A to GLC1F loci. Herein, we report identification of a new POAG locus (GLC1H) at the 2p15-p16 region.

METHODS

FAMILY INFORMATION

The POAG family panel used in this study was originally ascertained through the glaucoma registry of King’s College Hospital and membership of the International Glaucoma Association in London, England. This registry maintains updated information on visual acuity,
tonometry and gonioscopy results, and visual fields. One Afro-Canadian (Jamaican) and 6 white families were included in this study.

Clinical diagnosis was made by local ophthalmologists specializing in glaucoma, except for the Jamaican family, who was examined at home. Diagnosis was based on IOP, visual field loss, and optic disc appearance as determined by our study consultant. Optic nerve damage, as indicated by notching of the optic disc; increased cup-disc ratio (>0.5); and characteristic visual field loss as measured by automated Humphrey perimetry, as well as open- and normal-angle appearance (measured by gonioscopy) and IOP values of 22 mm Hg or higher while not taking medication, provided the basis for diagnosis of POAG. Subjects with an IOP of 22 mm Hg or lower were classified as having normal-tension glaucoma. Subjects with IOP values of 22 mm Hg or higher and no other signs of glaucoma were considered to have ocular hypertension (OH). All secondary cases of glaucoma were excluded. Most study subjects have been examined repeatedly over the years and their disease progression, history of ocular operations, and other treatment parameters were recorded at each visit. A standardized examination sheet was filled out by the glaucoma specialist and transferred to our glaucoma database.

GENOTYPING

Genomewide scans of 322 subjects with adult-onset POAG from 91 unrelated families revealed 13 new putative locations. Saturation mapping with additional polymorphic markers showed consistent linkage to 2p15-p16 only in 7 families. One large family consisted of 113 members and included 12 affected members and 1 obligate gene carrier (Figure 1 and Figure 2). Of these, 70 subjects were alive including 9 affected subjects and 1 subject with OH. A total

![Figure 1. Entire pedigree structure of the main family with primary open-angle glaucoma (POAG) with 12 affected subjects in 3 generations.](image-url)
of 49 subjects from 3 different generations ranging from 33 to 87 years of age, including 9 affected members and 1 subject with OH, were sampled. These were subsequently genotyped together with 6 other families (Figure 3) for 9 DNA markers from a region of approximately 27 centimorgans (cM). We further genotyped 16 subjects of the main family for a total of 15 DNA markers. Haplotypes were manually constructed and inspected for genotypic inconsistencies and coinheritance with the glaucoma phenotype (Figure 2).

**LINKAGE ANALYSIS**

Two-point linkage analysis was performed for 15 markers mapping to the 2p12-p16 region (Figure 2) by using the MLINK module of the LINKAGE package. The normal subjects in the fourth generation (Figure 1) were not included in the analysis because they were too young, well below the age at onset, and did not contribute to logarithm of the odds (LOD) scores. Linkage analysis was completed for 9 short tandem repeat polymorphism markers in 7 families that collectively provided a total of 85 informative meioses including 35 affected subjects, 32 normal subjects, and 18 asymptomatic gene carriers. To estimate more realistic LOD score values and to ascertain a better linkage relationship of POAG in these families, the uncertain disease status of the normal subjects and gene carriers were coded in 3 different ways, as discussed later.

**RESULTS**

**DISEASE PHENOTYPE IN THE 7 FAMILIES**

Age at diagnosis in the 7 families varied from 32 to 78 years (mean, 58 years). The maximum IOP values varied from moderate (24 mm Hg) to high (60 mm Hg). The affected subjects had optic nerve damage varying from early to late stages, with cup-disc ratios of up to 0.9 and significant glaucomatous visual field loss.

**DISEASE PHENOTYPE IN THE MAIN FAMILY**

The detailed clinical phenotype of the main family is presented in **Table 1**. The age at diagnosis varied from 48 to 78 years (mean, 64 years). The IOP values varied from 18 to 36 mm Hg (mean, 26 mm Hg). Optic nerve damage varied from early to late stages, with a cup-disc ratio of up to 0.9 and characteristic visual field loss. Nine of the 10 subjects with POAG took medication or underwent surgical treatment to lower their IOPs.

The haplotypes for the main family are presented in Figure 2. Two subjects did not show a definitive visual field loss. One subject (subject III-6) had an IOP of...
30 mm Hg; cup-disc ratios of 0.6 and 0.6; and inconsistent, abnormal visual field test results and she had her lens removed. The other subject (subject III-16) had IOPs of 34 and 25 mm Hg and cup-disc ratios of 0.6 and 0.6 and was taking both betaxolol hydrochloride and dorzolamide hydrochloride to lower her IOP. Another subject (subject II-7) living in isolation was not available for routine and follow-up examinations, but he is known to have had OH before he died.

HAPLOTYPE ANALYSIS

Construction and inspection of haplotype transmission data in the main family revealed a common affected haplotype expanding from D2S123 to D2S2165, a region of approximately 7 cM or 11 Mb (Figure 2). The affected haplotype was inherited by a minimum of 8 affected subjects in the third generation (aged 72-89 years) and 1 affected subject in the fourth generation (aged 55 years), who was diagnosed at the age of 48 years. The affected haplotype was transmitted from their mother (subject II-6); thus, she is considered an obligate gene carrier. Additionally, her brother (subject II-7) and her niece (subject III-16), born to an affected sister (subject II-16), carry the exact same haplotype from D2S156 to D2S329. Both of them share a part of the affected haplotype from D2S2156 to D2S2332 with subject II-6 (her haplotype was inferred from her 10 genotyped children). Therefore, it is realistic to assume that subjects II-7 and III-16 carry an extended affected haplotype, whereas subject II-6 carried a shorter recombinant haplotype centromeric to D2S2165; thus, all of her affected children inherited the same recombinant haplotype from her. The upper limit of the GLC1H locus is with D2S123 as determined by a recombination in subject III-3. Therefore, the GLC1H critical region lies between D2S123 and D2S2165, a region of 7.08 cM or approximately 10.8 Mb (Figure 2). All affected members of the other 6 families shared similar haplotypes that well overlapped with the main family but with no founder effect. Therefore, these are probably linked to

Figure 3. Pedigree structures of 6 additional families (pedigrees A-F) potentially linked to the GLC1H locus. DNA indicates that blood samples were taken and the extracted DNA used for linkage analysis; POAG, primary open-angle glaucoma.
the same region. However, 1 family (Figure 3, pedigree D) had an affected recombination with D2S2352, thus potentially narrowing the upper limit of this locus from D2S123 to D2S2352 or by approximately 2.59 Mb.

**LINKAGE ANALYSIS**

Genotyping of 15 polymorphic markers from the 2p12-p16 region in 16 subjects, including 9 affected subjects and 1 subject with OH (Figure 2), provided a maximum LOD score (Zmax) of 2.97 with D2S370 in the main family alone (Table 2).

When the 7 families were combined, 3 different analytical methods were used. The affected status was unchanged, but gene carriers and normal subjects were coded differently (Table 3). When normal and affected haplotype-carrying subjects (ie, gene carriers) were coded as “normal” (method 1), a Zmax of 3.96 at 18 cM was obtained with D2S2320. When normal subjects were coded as “normal” but gene carriers were coded as “unknown” (method 2), a Zmax of 9.30 at 4 cM was obtained with D2S2320. Lastly, when only affected subjects were used in the analysis and genotypic data for normal and gene carriers were deleted (method 3), a Zmax of 2.65 at 5 cM was obtained with D2S2320. We considered method 2, with a Zmax of 9.30 for D2S2320, as the most likely representation of this linkage data. The other methods probably signify the 2 extremes and less likely scenarios. However, all of these 3 methods provided sufficient evidence for linkage of a putative POAG locus to this region of chromosome 2 and further demonstrated both power and limitation of family-based linkage analysis.

<table>
<thead>
<tr>
<th>DNA Marker</th>
<th>Genetic Distance From Top (cM)</th>
<th>Obtained LOD Score Values at Various Recombination Fractions, cM</th>
<th>Peak LOD Value</th>
<th>Peak Recombination Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>D2S2156</td>
<td>73.61</td>
<td>51.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D2S122</td>
<td>73.61</td>
<td>51.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D2S1364</td>
<td>77.97</td>
<td>57.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D2S2315</td>
<td>80.16</td>
<td>59.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D2S406</td>
<td>80.16</td>
<td>59.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D2S1337</td>
<td>80.16</td>
<td>59.22</td>
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</tr>
<tr>
<td>D2S370*</td>
<td>80.16</td>
<td>59.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D2S444</td>
<td>80.16</td>
<td>60.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D2S2160</td>
<td>80.69</td>
<td>60.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D2S2332</td>
<td>80.69</td>
<td>61.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D2S2165</td>
<td>80.69</td>
<td>61.93</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D2S2320</td>
<td>82.29</td>
<td>62.81</td>
<td></td>
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</tr>
<tr>
<td>D2S441</td>
<td>86.82</td>
<td>67.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D2S1374</td>
<td>90.82</td>
<td>72.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D2S329</td>
<td>101.02</td>
<td>79.13</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: cM, centimorgan; LOD, logarithm of the odds; Mb, megabase; ∞, infinity.

*This DNA marker produced the highest LOD score.

Table 1. Detail Clinical Information of the Main Family Used for Linkage Analysis

<table>
<thead>
<tr>
<th>Subject*</th>
<th>Age at Diagnosis, Y</th>
<th>Highest IOP, mm Hg</th>
<th>Visual Field Loss</th>
<th>Cup-Disc Ratio</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>III-3</td>
<td>78</td>
<td>22</td>
<td>Yes</td>
<td>0.8</td>
<td>Levozunol hydrochloride and dorzolamide hydrochloride administered</td>
</tr>
<tr>
<td>III-6</td>
<td>71</td>
<td>30</td>
<td>unknown†</td>
<td>0.6</td>
<td>Lens removed</td>
</tr>
<tr>
<td>III-7</td>
<td>77</td>
<td>24</td>
<td>Yes</td>
<td>0.6</td>
<td>None</td>
</tr>
<tr>
<td>III-8</td>
<td>62</td>
<td>19‡</td>
<td>Yes</td>
<td>0.8</td>
<td>Levozunol administered</td>
</tr>
<tr>
<td>III-9</td>
<td>69</td>
<td>39</td>
<td>Yes</td>
<td>0.7</td>
<td>Levozunol and pilocarpine administered</td>
</tr>
<tr>
<td>III-10</td>
<td>66</td>
<td>26</td>
<td>Yes</td>
<td>0.7</td>
<td>Trabeculectomy</td>
</tr>
<tr>
<td>III-12</td>
<td>54</td>
<td>24</td>
<td>Yes</td>
<td>0.7</td>
<td>Brimonidine tartrate administered, trabecuoplasty</td>
</tr>
<tr>
<td>III-13</td>
<td>68</td>
<td>unknown</td>
<td>Yes</td>
<td>0.8</td>
<td>Latanoprost and brimonidine administered</td>
</tr>
<tr>
<td>III-16</td>
<td>51</td>
<td>34</td>
<td>No</td>
<td>0.6</td>
<td>Betaxolol hydrochloride and dorzolamide administered</td>
</tr>
<tr>
<td>IV-2</td>
<td>48</td>
<td>18</td>
<td>Yes</td>
<td>0.9</td>
<td>Brimonidine and dorzolamide administered; cataract removed</td>
</tr>
</tbody>
</table>

Abbreviation: IOP, intraocular pressure.

*Subject numbers from Figure 2.
†Abnormal visual field test results.
‡While taking medication, otherwise unknown.

Table 2. Two-Point LOD Scores Between the GLC1H Locus and 15 DNA Markers in the Main Family

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Use of genomic convergence and proteomic streamlining methods identified a number of promising candidates. Therefore, if we were to combine the data from these 2 families, the clinical manifestation in affected subjects of these families showed classic adult-onset glaucoma with cupping of the optic nerve head, visual field loss, and medium to high elevated IOP. The mean age at diagnosis in the main family varied from 48 to 78 years (mean, 64 years), and in other families, it ranged from 32 to 78 years (mean, 58 years). Therefore, a large number of normal subjects were too young for inclusion in our statistical linkage analysis.

The GLC1H critical region contains 61 known genes. Use of genomic convergence and proteomic streamlining methods identified a number of promising candidate genes based on their ocular expression or function. So far, we have screened 35 of these genes but have not found a causative mutation. By using GMP Conversion Technology (GMP Genetics, Inc, Waltham, Mass), we made specific cell lines carrying only a normal- or affected-bearing chromosome of the proband (subject III-12 in Figure 2). The generated genomic and complementary DNA of these 2 cell lines are currently being used for mutation screening of other genes from within the GLC1H region.

In summary, a new POAG locus (GLC1H), as established by extensive haplotype and linkage analysis of a very large pedigree, is linked to a region of approximately 8.3 Mb on 1p15-p16. Cloning of the GLC1H defective gene could help early diagnosis and personalized treatment for this late-onset condition.

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### Table 3. Combined 2-Point LOD Scores for DNA Markers in 7 Families With POAG*

<table>
<thead>
<tr>
<th>DNA Markers Studied</th>
<th>Mb From Top of Chromosome 2</th>
<th>Genetic Interval, Mb</th>
<th>Method 1‡</th>
<th>Method 2‡</th>
<th>Method 3§</th>
</tr>
</thead>
<tbody>
<tr>
<td>D2S122</td>
<td>51.55</td>
<td>0.00</td>
<td>0.49</td>
<td>4.11</td>
<td>1.88</td>
</tr>
<tr>
<td>D2S2352</td>
<td>53.73</td>
<td>2.18</td>
<td>1.25</td>
<td>7.69</td>
<td>1.80</td>
</tr>
<tr>
<td>D2S1364</td>
<td>57.44</td>
<td>3.71</td>
<td>1.86</td>
<td>6.77</td>
<td>2.04</td>
</tr>
<tr>
<td>D2S2332</td>
<td>62.91</td>
<td>5.47</td>
<td>3.96</td>
<td>9.30</td>
<td>2.65</td>
</tr>
<tr>
<td>D2S1772</td>
<td>66.91</td>
<td>4.00</td>
<td>1.21</td>
<td>5.12</td>
<td>1.52</td>
</tr>
</tbody>
</table>

Abbreviations: cm, centimorgan; LOD, logarithm of the odds; Mb, megabase; POAG, primary open-angle glaucoma; Zmax, maximum LOD score.

‡For method 1, 35 affected subjects were coded as “affected”; 32 normal subjects, as “normal”; and 18 gene carriers, as “normal.”

§For method 3, 35 affected subjects were coded as “affected”; 32 normal subjects, as “deleted”; and 18 gene carriers, as “deleted.”

### Comment

Saturation mapping of the 1.6p12-p16 region as originally identified by a genomewide scan showed promising genetic linkage in 7 families with adult-onset POAG. Haplotype analysis of a large family with 9 affected members indicated that the disease in this family is linked to 8 DNA markers from D2S1364 to D2S2332 (Figure 2), with a Zmax ranging from 2.35 to 2.97 at θ=0, the highest being 2.97 for D2S370 (Table 2). Assuming a minimum number of recombinational events that occur in the transmission of traits from 1 generation to another, detailed examination of the inherited haplotypes in the main family (Figure 2) suggested that an obligate gene carrier (subject II-6) was recombinant for D2S2165 and all other centromeric markers rather than her 2 other relatives (subjects II-7 and III-16). According to this large family, the critical region of the GLC1H locus lies between D2S123 and D2S2165, a region of 7.08 cm that corresponds to approximately 10.8 Mb. Another family (Figure 3, pedigree D) had an affected crossover with D2S2352. Therefore, if we were to combine the data from these 2 families, the GLC1H locus maps within a 4-cM region between D2S2352 and D2S2165, a physical distance of about 8.3 Mb. Combined analysis of the 7 families produced the highest LOD score of 9.30 for the DNA marker of D2S3230 (Table 3, method 2), which is approximately 3 Mb centromeric to D2S370 (Zmax=2.97), as obtained for the main family (Table 2).

The clinical manifestation in affected subjects of these families showed classic adult-onset glaucoma with cupping of the optic nerve head, visual field loss, and medium to high elevated IOP. The mean age at diagnosis in the main family varied from 48 to 78 years (mean, 64 years), and in other families, it ranged from 32 to 78 years (mean, 58 years). Therefore, a large number of normal subjects were too young for inclusion in our statistical linkage analysis.

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In summary, a new POAG locus (GLC1H), as established by extensive haplotype and linkage analysis of a very large pedigree, is linked to a region of approximately 8.3 Mb on 1p15-p16. Cloning of the GLC1H defective gene could help early diagnosis and personalized treatment for this late-onset condition.

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### References


**Correction**

Error in Author Name. In the Correspondence letter titled “Effects of Intravitreous Injection of Preserved and Non-preserved Triamcinolone in Rabbit Retina,” published in the November 2006 issue of the *ARCHIVES* (2006;124:1666), there was an error in the second author’s name. It should have read, “Muhammad M. Abd-El-Barr, MS.”