A Genome-wide Scan Maps a Novel Juvenile-Onset Primary Open-Angle Glaucoma Locus to 15q

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PURPOSE. To map the disease-associated locus of a family with autosomal dominant juvenile-onset primary open-angle glaucoma (JOAG).

METHODS. A complete ophthalmic examination was conducted, and genomic DNA was obtained from 25 members of a Chinese family, of which eight were confirmed as having JOAG. Myocilin (MYOC), optineurin (OPTN), and WD repeat-domain 36 (WDR36) were screened for sequence alterations, by PCR and direct sequencing. Subsequently, a genome-wide scan was performed (Prism Linkage Mapping Set MD-10; Applied Biosystems, Inc., Foster City, CA). Two-point and multipoint linkage analyses were performed with the MLINK, ILINK, and LINKMAP programs. For fine mapping, additional markers flanking the most promising region on 15q were analyzed. The significance of the lod score was tested with simulation analyses by using FASTLINK. Haplotypes were constructed with Simwalk2. Three candidate genes, NR2E3, SMAD6, and CLN6, located within the critical region, were screened for mutations.

RESULTS. MYOC, OPTN, and WDR36 mutations were excluded in all family members. A maximum two-point lod score of 3.31 at \( \theta = 0.0 \) was obtained for the marker D15S125. Four adjacent markers, rs2030040, rs169169963, D15S153, and D15S131, gave two-point lod scores of 2.41, 2.90, 3.02, and 2.68, respectively, at \( \theta = 0.0 \). Haplotype analysis and recombination mapping further confined this region to 15q22-q24 within a genetic distance of 16.6 Mb flanked by D15S1036 and rs922693. No mutations were found in the coding exons and splicing junctions of NR2E3, SMAD6, and CLN6.

CONCLUSIONS. The results provide evidence for the mapping of a novel locus for JOAG at 15q22-q24. Further search for the disease-causing gene in this new JOAG locus is in progress.

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q14 in a study that involved eight Finnish POAG families, indicating that additional loci are involved in the etiology of POAG.41 Four genes (PIX2, FOXC1, PAX6, and CYP1B1) have been reported to cause JOAG as well as Axenfeld-Rieger syndrome, aniridia, or congenital glaucoma.42–44 In the present study, we performed a genome-wide scan and identified a novel locus to 15q22-q24 in one JOAG family, supported by clinical, linkage, and haplotype transmission data.

METHODS

Pedigree Structure

One three-generation family segregating JOAG was recruited from Hong Kong Eye Hospital, Hong Kong (Fig. 1). One experienced ophthalmologist (JKHC) conducted complete ophthalmic examinations, including visual acuity, slit-lamp examination, gonioscopy, applanation tonometry, ophthalmoscopy with evaluation of the optic disc, and visual field testing (Humphrey Visual Field Analyzer; Carl Zeiss Meditec, Inc., Dublin, CA) in 25 of the 31 members of this pedigree (Fig. 1). Peripheral venous blood from these subjects was collected for genomic DNA extraction. The other six family members (II:2, II:3, II:5, II:8, II:10 and II:12), who were spouses of some offspring of I:1 and I:2, were not willing to come for eye examinations and blood sampling, but they gave informed consent for clinical information to be retrieved from their medical records. JOAG was diagnosed if patients demonstrated the following characteristics: exclusion of secondary causes (e.g., trauma, uveitis, or steroid-induced glaucoma), onset of the disease before age 35 years, anterior chamber angle open (grade III or IV gonioscopy), IOP equal to or greater than 22 mm Hg in both eyes, glaucomatous optic disc damage in both eyes and typical visual field loss in at least one eye. Clinical features of all family members affected with JOAG at the time of recruitment in 1999 are listed in Table 1. In 2006, another experienced ophthalmologist (CKSL) conducted the same complete ophthalmic examinations as well as optical coherence tomography (OCT) imaging (StratusOCT, version 3; Carl Zeiss Meditec, Inc.) of all 25 family members. The study protocol was approved by the Ethics Committee for Human Research of the Chinese University of Hong Kong. In accordance with the tenets of the Declaration of Helsinki, informed consent was obtained from all family members after explanation of the nature and possible consequences of the study.

Genotyping

MYOC, OPTN, and WDR36 were screened for sequence alterations by PCR and direct sequencing, as previously reported.29,30,46 A genome-wide scan was performed with a linkage mapping set (Prism Linkage Mapping Set MD-10; Applied Biosystems, Inc. [ABI], Foster City, CA) comprising 400 microsatellite markers with an average spacing of 10 cM. The amplified PCR products were run on a DNA sequencer (model 377; ABI). Data collection and genotyping were performed by software accompanying the system (The GeneScan and GenoTyper; ABI). Two other packages (GenoPedigree and GenoBase; ABI) were used to draw the pedigree and to export data for linkage analysis. For fine mapping, an additional 13 microsatellite markers and 29 single-nucleotide polymorphisms (SNPs) flanking the promising region were analyzed in a similar fashion.
After a linkage of 15q22-q24 was established, three genes within the critical region including \( NR2E3 \), \( SMAD6 \), and \( CLN6 \), were selected for mutation screening. The selection of these genes was based on their function, possibly related to glaucoma and their expression in eye tissues. The respective coding exons and splice junctions were amplified by PCR and screened for sequence alterations by direct DNA sequencing. In addition, up to 1700 bp of the promoter region of \( NR2E3 \) was screened for sequence alterations.

### RESULTS

#### Phenotypes of the Patients

Ages of diagnosis for the members of this family with glaucoma ranged from 12 to 32 years (Table 1; median 22, mean 22.25). All these affected family members showed notable increase in IOP and severe visual loss. The IOP of these patients could not be controlled with available antiglaucoma medication. They all had a wide anterior chamber angle. Although most of them had typical glaucoma changes in optic disc and visual field, they all showed a normal iris, no anterior segment anomalies, and corneal thickness and anterior chamber depth within normal ranges. This pedigree was therefore considered a JOAG family on the basis of typical features of POAG and age of diagnosis younger than 35 years. Clinical information and segregation analysis showed an autosomal dominant inheritance (Fig. 1, Table 1). Recent complete ophthalmic examination and OCT imaging on the same 25 family members (i.e., 7 years after the examination at recruitment in 1999), revealed no additional changes in clinical features. The only exception was subject II:1, who showed some visual field loss change. He was 70 years old and was therefore ruled out for JOAG.

#### Exclusion of \( MYOC \), \( OPTN \), and \( WDR36 \)

We screened a total of 25 family members (8 with JOAG) for sequence alterations in the coding regions and splice junctions.
of MYOC, OPTN, and WDR36. No disease-causing mutations were identified. Only three polymorphisms in MYOC (−83G→A, R76K, and IVS2+35A→G), five polymorphisms in OPTN (M98K, IVS6-10G→A, IVS6-5T→C, IVS8+81C→A, and IVS15-48C→A), and two polymorphisms in WDR36 (IVS16-30A→G, V727V) were found in this family (Table 2). None of these polymorphisms segregated with JOAG and thus were not associated with glaucoma in this family.

**Genome-wide Scan**

After exclusion of MYOC, OPTN, and WDR36 as disease-causing genes in this family, a genome-wide search was performed using the maker set (MD-10; ABI). To increase the statistical power, only 23 informative meioses were used in our linkage analysis (Fig. 1). Among them, there were eight patients with JOAG. The microsatellite markers were spaced on a 10-cM average grid on all chromosomes. After the first scan, markers adjacent markers at loci D15S978, D15S1007, D15S1009, and D15S1012 that cover the whole region of GLC1J, showed negative lod scores (Table 3). It was noteworthy that markers located on chromosomes 1, 9, and 10 were not in the previously reported POAG loci (GLC1A, GLC1J, and GLC1E). However, these three chromosomal regions were not further pursued because of the insignificant lod scores.

### Fine Mapping

Thirteen additional microsatellite markers and 29 SNP markers were genotyped for fine mapping: D15S1028, D15S161, D15S1032, D15S1016, D15S198, D15S1033, D15S1036, rs2030040, rs242379, rs11071720, rs1126308, rs11857261, rs1043256, rs16949963, rs7162473, rs12900990, rs3816385, rs8032157, rs16948162, rs3743046, rs16948311, rs12915851, D15S1009, D15S125, D15S1041, rs3971872, rs922693, D15S1023, rs894785, rs7183668, rs7183127, rs894783, rs2304991, rs3816282, rs745029, rs2870433, rs1685163, rs419020, rs2586179, rs11638180, D15S1005, and D15S211. They were spaced, on average, 0.8 Mb (0.0-7.9 Mb) apart. Except for 14 uninformative SNP markers, linkage analysis of the other 28 markers gave positive lod scores (Table 5). A maximum two-point lod score of 3.31 at \( \theta = 0.00 \) was obtained for D15S125. Multi-point analysis of these markers did not increase the highest lod score. When only affected meioses were used in two-point linkage analysis, the maximum lod score was reduced to 1.81 at \( \theta = 0.00 \) for D15S125. We used FASTLINK to generate 10,000 unlinked replicates with allele frequencies of D15S125 and then computed the best lod score for each replicate. No replicates exceeded the true lod score of 3.31 and gave an empirical \( P < 0.0001 \).

### Haplotype Construction

Eleven microsatellite or SNP markers within the flanking region of D15S1033 and D15S211 were used to construct the haplotypes (Fig. 1). Inspection of the haplotype transmission data identified a common disease haplotype that has been clearly inherited by all eight affected subjects (I:1, II:4, II:6, II:9,II:13, II:15, III:4 and III:6) and five other at-risk subjects at 20 (III:3), 17 (III:5), 16 (III:10), 14 (III:12) and 10 (III:13) years of age at the time of study. These five phenotypically normal subjects carried the disease haplotype, but were still too young to show any sign of glaucoma and may develop glaucoma at some time in future. Further inspection of the haplotypes in this pedigree

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**Table 3. Markers with Two-Point Lod Scores >1.0 in a Genome-wide Scan**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Location</th>
<th>Two-point LOD Scores at ( \theta = 0.00 )</th>
<th>( Z_{\text{max}} )</th>
<th>( \theta_{\text{max}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>D15S978</td>
<td>15q21</td>
<td>1.05 1.02 0.91 0.78 0.50 0.24 0.06 0.00</td>
<td>0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00</td>
<td>0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00</td>
</tr>
<tr>
<td>D15S1007</td>
<td>15q23</td>
<td>3.02 2.98 2.78 2.52 1.95 1.31 0.58 0.00</td>
<td>0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00</td>
<td>0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00</td>
</tr>
<tr>
<td>D15S1012</td>
<td>15q23</td>
<td>2.68 2.64 2.47 2.23 1.73 1.16 0.50 0.00</td>
<td>0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00</td>
<td>0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00</td>
</tr>
</tbody>
</table>

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**Table 4. Two-Point Lod Scores for Markers Flanking the Reported GLC1J Locus**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Sequence Map (Mb)</th>
<th>Marshfield Map (cM)</th>
<th>Two-point LOD Scores at ( \theta = 0.00 )</th>
<th>( Z_{\text{max}} )</th>
<th>( \theta_{\text{max}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>D15S128</td>
<td>22.60</td>
<td>6.11</td>
<td>−1.14 −1.07 −0.82 −0.58 −0.28 −0.12 −0.03 −0.03</td>
<td>0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04</td>
<td>0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04</td>
</tr>
<tr>
<td>D15S1002</td>
<td>25.52</td>
<td>14.58</td>
<td>−0.81 −0.95 −0.75 −0.54 −0.27 −0.11 −0.03 −0.03</td>
<td>0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04</td>
<td>0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04</td>
</tr>
<tr>
<td>D15S165</td>
<td>29.04</td>
<td>20.24</td>
<td>−0.84 −0.04 −0.05 −0.06 −0.07 −0.05 −0.02 −0.02</td>
<td>0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04</td>
<td>0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04</td>
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<tr>
<td>D15S1007</td>
<td>31.55</td>
<td>25.86</td>
<td>−0.03 −0.03 −0.04 −0.05 −0.06 −0.04 −0.01 −0.01</td>
<td>0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04</td>
<td>0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04</td>
</tr>
<tr>
<td>D15S1012</td>
<td>36.79</td>
<td>35.95</td>
<td>−0.03 −0.03 −0.04 −0.05 −0.06 −0.04 −0.01 −0.01</td>
<td>0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04</td>
<td>0.04 0.04 0.04 0.04 0.04 0.04 0.04 0.04</td>
</tr>
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</table>
revealed two critical recombination events in two affected individuals (II:6 and III:4) that limited the location of the JOAG locus telomeric to rs922693 and centromeric to D15S1036, within a genetic distance of 16.6 Mb (Figs. 1, 2). This region was located on 15q22-q24.

**Candidate Gene Search**

Within the 15q22-q24 region, 118 genes have been annotated in the NCBI Map Viewer (Build 36). After a search for the information on gene function in this region, we found that NR2E3, CLN6, and SNAAD6 are the most probably related to glaucoma. All of them were expressed in the retina. However, our mutation screening of these three genes revealed no disease-causing mutations in this family. Only four polymorphisms in NR2E3 (−1052G→A, −169C→G, E140G, and M163T) and three polymorphisms in SNAAD6 (A149A, IVS2-22C→A, and F398F) were found in the family members. No sequence variations in CLN6 were detected in any of the family members. Two polymorphisms, −1052G→A in NR2E3 and A149A in SNAAD6, gave two-point lod scores of 1.24 at θ = 0.00 and 1.79 at θ = 0.10, respectively, providing further evidence on positive linkage at this new JOAG locus.

**DISCUSSION**

In the present study, we provide evidence for a novel locus that should harbor a gene responsible for JOAG, other than MYOC, OPTN, and WDR36. In a genome-wide scan, we de-
ected a significant linkage with DNA markers at 15q. Fine mapping with additional markers within this region supported the linkage. Haplotype analysis and recombination mapping further confirmed the critical region to 15q22-2q4 within a genetic distance of 16.6 Mb flanked by markers D15S1036 and rs922693. To date, more than 20 genetic loci have been reported for POAG.11 However, only three of them were linked to JOAG, including GLC1A on 1q23-q25, GLC1J on 9q22, and GLC1K on 20p12, whereas the others contributed only to adult-onset POAG. All these three JOAG loci were exclusively identified in white persons. Recently, we mapped the fourth JOAG locus to 5q22.1q32 in a large autosomal dominant JOAG family from the Philippines.46 In the present study, we further mapped the fifth JOAG locus to 15q22-2q4 in a Chinese JOAG family. Our previous mutation screening of three known POAG genes (MYOC, OPTN, and WDR36) has demonstrated a different mutation pattern in Chinese than in whites.29-30,46 Our findings of the two new JOAG loci in Asians further indicate that the genetic basis of JOAG in Asians may be very different from that in whites.

Recently Allingham et al.26 identified an early adult-onset POAG locus at 15q11-q13, designated GLC1I. The maximum lod score was 3.24 (P = 0.013 by permutation) at GABRB3 which is located at approximately 9 cM on the Marshfield map (research.marshfieldclinic.org.genetics/provided in the public domain at Marshfield Clinic, Marshfield, WI). Further evidence for GLC1I as a locus of early adult-onset POAG was given in an independent study on 25 multiplex POAG families.22 The maximum lod score was 2.09 (P = 0.021) at 26.1 cM on chromosome 15. In the present study, our results support the mapping of a novel JOAG locus at 15q22-2q4, which is 55.23 or 38.1 cM apart from the GLC1I locus. In addition, all five ABI MD-10 microsatellite markers (D15S102, D15S102, D15S165, D15S107, and D15S1102), which locate at 6 to 35 cM on the Marshfield map and cover the whole region of GLC1I showed negative lod scores in our JOAG family. Their haplotypes did not segregate with glaucoma phenotype.

In the 15q22-2q4 region, several genes—notably, NR2E3, SMAD6, and CLN6—possess properties indicative of association with glaucoma. NR2E3 (OMIM 604485; Online Mendelian Inheritance in Man; http://www.ncbi.nlm.nih.gov/Omim/ provided in the public domain by NCBI, Bethesda, MD) encodes a retinal nuclear receptor that is a potential regulator of cellular retinaldehyde-binding protein expressed in retinal pigment epithelium in Müller glial cells.30-31 SMAD6 (OMIM 602951) is expressed predominantly in heart and blood vessels and plays a role in the homeostasis of cardiovascular system. SMAD6 variants have been linked to the development of aortic ossification and elevated blood pressure.32 CLN6 (OMIM 604485) is a causative gene of variant late infantile neuronal ceroid lipofuscinosis, which is a heterogeneous group of autosomal recessive neurodegenerative disease.33 CLN6 is expressed in adult and embryonic brains in mice and humans. However, after screening the coding exons and splicing regions of NR2E3, SMAD6, and CLN6, we did not find any sequence alterations in our study subjects. Our experience on this type of candidate gene approach has been that it is not efficient and depends too much on chance. We therefore attempted an alternative approach for better efficiency and effectiveness in identifying new disease-causing genes in identified linkage loci. The principle is basically similar to that of genome-wide association study,54 but our intention is not to investigate the whole genome but a small region of the genome—for example, 15q22-2q4, where the new JOAG locus was mapped. To do that, we have selected more than 100 gene-based SNPs within 15q22-2q4, roughly one SNP for one gene. We intend to genotype these SNPs in a group of 100 unrelated patients with JOAG and another group of 100 unrelated control subjects. This approach should enable us to search exhaustively for glaucoma-associated genes in the new JOAG locus.

From the haplotype data we identified five at-risk subjects in this family: III:3, III:5, III:10, III:12, and III:13, current age, 31, 22, 21, 20, and 16 years, respectively. They still showed no obvious glaucoma features after OCT and a repeated complete ophthalmic examination. All these subjects, together with other family members of the same generation (III:1, III:2, III:4, III:6, III:7, III:8, III:9, and III:11) will attend a follow-up plan of eye examination including anaplanation tonometry, funduscopy, and perimetry every 6 months. The remainder of the family members will be followed up every year.

In conclusion, we provide evidence of new JOAG locus mapped to 15q22-2q4. Our present findings and other reports support JOAG as a complex genetic disease. Identification of genes contributing to the variance of this disorder will enhance our understanding of the pathophysiology of JOAG as a whole.

References


