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A genome-wide scan maps a novel juvenile-onset primary open angle glaucoma locus to chromosome 5q

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Purpose: To map the disease-associated locus of a family with autosomal dominant juvenile-onset primary open angle glaucoma (JOAG) and to screen the novel glaucoma gene *WD repeat domain 36* (*WDR36*).
Methods: Complete ophthalmic examination and genomic DNA were obtained from 27 family members, in which nine were confirmed JOAG patients. *Myocilin* (*MYOC*), *optineurin* (*OPTN*), and *WDR36* were screened for mutations by polymerase chain reaction and direct sequencing. Genome-wide scanning was carried out using the ABI PRISM Linkage Mapping Set MD-10. 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 chromosome 5q were also analyzed. The significance of LOD scores was tested with simulation analyses using FASTLINK. Haplotypes were constructed using Simwalk2.
Results: *MYOC* or *OPTN* mutations were excluded in all family members. A maximum LOD score value of 4.82 at θ=0.00 was obtained for the marker D5S2011. Markers D5S2065, D5S1384, D5S471, D5S503, D5S2098, and D5S638 had LOD score values over 4.0 at θ=0.00. Haplotype analysis and recombination mapping further confined this region to 5q22.1-q32 within a region of 36 Mb flanked by D5S2051 and D5S2090. Screening of the novel *WDR36* glaucoma-associated gene, which lies centromeric to the disease interval, revealed no mutations within any of the 23 coding exons or splicing junctions.
Conclusions: Our results provided the mapping of a novel locus for JOAG at 5q and excluded coding or splicing junctions mutations within the *WDR36* gene.

Glaucoma is a leading cause of vision impairment and blindness in both developed and developing countries. Primary open angle glaucoma (POAG) occurs most frequently, accounting for more than 50% of all cases of the disease [1]. Juvenile-onset primary open angle glaucoma (JOAG) is a subset of POAG that appears earlier in life and is often inherited in an autosomal dominant manner [2].

POAG is a genetically heterogeneous disorder, but the molecular basis of most cases remains unknown. Seven chromosomal loci have been linked with POAG: 1q23 (*GLC1A*) [3], 2cen-q13 (*GLC1B*) [4], 3q21-q24 (*GLC1C*) [5], 8q23 (*GLC1D*) [6], 10p15-p14 (*GLC1E*) [7], 7q35-q36 (*GLC1F*) [8], and 5q22.1 (*GLC1G*) [9]. A genome-wide scan involving an initial pedigree set of 113 affected sib-pairs and a second pedigree set of 69 affected sib-pairs has reported additional association to regions 2p14, 14q11, 14q21-q22, 17p13, 17q25, and 19q12-q14 [10]. Another genome-wide scan on 146 POAG families of African descent suggested possible linkage to 2q33.3-q37.3 and 10p12-p13 [11]. A recent genome-wide scan revealed putative glaucoma loci on 9q22 and 20p12 in 25 families with juvenile-onset POAG [12]. Among these loci, only three of them (1q21-q31, 9q22, and 20p12) contributed to JOAG while the others exclusively accounted for adult-onset POAG [3,12]. However, none of these loci showed linkage with POAG in a study of eight Finnish POAG families [13].

To date, only three genes have been identified from these loci. The first gene identified from *GLC1A* is *myocilin* (*MYOC*; OMIM 601652) [14]. In Caucasians, about 2-4% of POAG cases are due to *MYOC* mutations [15-17], although it can be as high as 36% in JOAG families [18]. In our previous studies, the prevalence of *MYOC* mutations was about 1.1-1.5% in Chinese POAG patients [19,20]. The second gene for POAG was identified from *GLC1E* as *optineurin* (*OPTN*; OMIM 602432) [21]. Mutations in *OPTN* were initially found in 16.7% of families with hereditary and adult-onset POAG and 12% of sporadic patients with POAG, the majority of them with intraocular pressure (IOP) of less than 22 mm Hg [21]. Two subsequent studies on Caucasian POAG patients reported no glaucoma-causing mutations in *OPTN*. One study involved

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801 patients of variable age onset [22] and the other had 86 adult-onset patients [23]. A study of 148 Japanese patients with normal tension glaucoma and 165 with POAG also found no specific glaucoma-causing mutations in *OPTN* [24]. We found *OPTN* mutations to account for 1.6% of sporadic POAG in Chinese patients [25]. The third gene for POAG was recently characterized as the *WD repeat domain 36* (*WDR36*; GenBank NM_139281) gene at *GLC1G* [9]. *WDR36* is a novel gene with 23 exons and encodes for a 951 amino acid protein with several WD40 repeats. In the original study reporting *WDR36* as the *GLC1G* gene, four mutations were found associated with more than 5% of all sporadic cases of POAG.

Besides the 17 aforementioned genetic loci, additional loci are expected to be involved in the etiology of this group of eye disorders. In this study, we present the chromosomal mapping of a novel locus to the 5q region in one JOAG family.

METHODS

Pedigree ascertainment: As previously reported a large family was recruited from the Ibanez region of the Philippines [26]. The study protocol was approved by the Ethics Committee for Human Research, the Chinese University of Hong Kong. In accordance with the tenets of the Declaration of Helsinki, informed consent was obtained from all living family members after explanation of the nature and possible consequences of the study. This five-generation family had a total of 95 members, in which 22 were affected with JOAG. Complete ophthalmic examinations were given to 27 family members, in which nine were confirmed JOAG patients. Peripheral venous whole blood from these subjects was collected for genomic DNA extraction. The other family members did not agree to participate in this study. Their clinical information was obtained through previous medical records. The incompleteness of the pedigree structure might pose a founder-effect that may increase the possibility of false-positive linkage. JOAG was defined as meeting all of the following criteria: exclusion of secondary causes (e.g., trauma, uveitis, or steroid-induced glaucoma), anterior chamber angle open (grade III or IV gonioscopy), an IOP greater than or equal to 22 mm Hg in both eyes by applanation tonometry, characteristic optic disc damage or typical visual field loss by Humphrey automated perimeter with the Glaucoma Hemifield Test, and diagnosis before age 35. Visual acuity was determined using Snellen eye chart. For the affected subjects, age at diagnosis ranged from 12 to 33 years (mean±SD: 19±4.2 years), the highest IOP from 24 to 44 mm Hg (mean±SD: 32±6.3 mm Hg), and cup to disc ratio from 0.7 to 0.9 (mean±SD: 0.8±0.04).

Genotyping: Genotyping was carried out using the ABI PRISM Linkage Mapping Set MD-10 (Applied Biosystems, Foster City, CA) comprising 382 microsatellite markers with an average spacing of 10 cM. Polymerase chain reactions (PCRs) were performed following the manufacturer's protocols in a Perkin-Elmer 9700 thermocycler (Applied Biosystems). The PCR products were subsequently pooled and separated on a 5% denaturing polyacrylamide gel in an ABI 377 DNA sequencer (Applied Biosystems). The Genescan and Genotyper software packages (Applied Biosystems) were used to call genotypes. The GenoPedigree and GenBase software packages (Applied Biosystems) were used to draw pedigree and to export data for linkage analysis. Family relationships were confirmed by observation of Mendelian inheritance of genotypes of microsatellite makers from all panels of the ABI Linkage Mapping Set MD-10 using PedCheck program [27], which also provided significant error-checking for the genotyping. For fine mapping, an additional 45 microsatellite markers flanking the promising region on chromosome 5 were analyzed in a similar fashion.

Linkage analysis: Two-point linkage analyses were performed with the MLINK and ILINK programs from the FASTLINK version 4.1P software package [28,29]. Regions containing markers that yielded LOD scores >3.0 were further analyzed by multipoint analyses using the LINKMAP program. According to Sarfarazi et al. [7], the JOAG gene frequency was set as 0.0001. An autosomal dominant mode of inheritance was used with one liability class with penetrance values 0, 1, 1, respectively, since no skipped generations were observed in this family. The significance of LOD scores was tested with simulation analyses using FASTLINK version 4.1P. Haplotypes for markers from the fine mapping regions were constructed using Simwalk2 version 2.83 [30].

Mutation screening: The *MYOC* and *OPTN* genes were screened for sequence alterations by PCR and direct sequencing as previously reported [19,25]. In addition, the *MYOC* promoter polymorphism, *MYOC*.mt1 (-1000C>G), was investigated by restriction endonuclease assays as previously reported [31]. The *WDR36* gene was screened as follows. Primers used to obtain the initial amplicons are given in Table 1. Initial PCRs were performed on an Applied Biosystems Gene Amp PCR System 9700 (96 wells, Applied Biosystems) in a total volume of 50 µl containing 100 ng of genomic DNA, 10 pmol of each primer, 200 µM dNTPs, 20 mM Tris-HCl (pH 8.0), 50 mM KCl, 1.5 mM $MgCl_2$, and 1 U of platinum Taq DNA polymerase (Invitrogen, Burlington ON). Cycling conditions were as follows: the first denaturation step of 5 min at 95 °C, 35 cycles of denaturation (95 °C for 30 s), annealing (56 °C for 30 s), elongation (72 °C for 30 s), and a final single elongation step of 7 min. PCR products were diluted in 5 volumes of PB buffer (Qiagen, Mississauga ON), transferred on a Whattman GF/C filter plate, washed twice with 80% ethanol/20 mM Tris (pH 7.5), and eluted in 50 µl of water. Samples were quantified by the PicoGreen reagent protocol. A second PCR was performed using the sequencing primers as described in Table 1 on an Applied Biosystems Gene Amp PCR System 9700 (96 wells) or 9700 Viper (384 wells) machines to incorporate the sequencing dyes (Big Dyes, version 3.1 from ABI), using a protocol of 25 cycles of denaturation (95 °C for 10 s) and annealing (55 °C for 5 s), followed by one last step of elongation (59 °C for 2 min). PCR products were purified by the ABI ethanol-EDTA precipitation protocol, collected using a Beckman-Coulter Allegra 6R centrifuge (Beckman-Coulter, Fullerton, CA), and resuspended in a 50% HiDi-formamide solution. Samples were then run on an Applied Biosystems Prism 3730xl DNA Analyzer automated sequencer. Sequence data were analyzed using the Staden preGap4 and Gap4 pro-

grams. Each amplicon was sequenced on one strand. As no variations or discrepancies were observed compared to the wild-type sequence, the complementary strand was not sequenced.

RESULTS

Pedigree structure: As previously reported [26], the five generations of vertical inheritance of the JOAG phenotype displayed a direct male-to-male transmission with similar num-

Table 1. Primers used in PCR and sequencing for WDR36

PCR primer	Primer sequence	Sequencing primer	Genomic position (NCBI build 35.1)	Amplicon size (bp)
1F	TTCTGTCGGAACCTAACGAGC	1F	110455756-110455776	517
1R	GAGTTAGAGGCCAAGGAGGG		110456253-110456272	
2F	AGTAAGTGTCTTTCTTATGAAGG	2F	110458388-110458410	263
2R	ATACATGTTACCTTGGCTTCC		110458630-110458650	
3F	GGACAAGGTGATTTCCTATCC	3R	110460468-110460489	473
3R	TTCTGAAAATTTATCTTCCTCCAG		110460917-110460940	
4F	GAGCAGATGAACATGCCTGG	4F	110462136-110462155	388
4R	CTCTGGCATAGATGTTTACATAG		110462501-110462523	
5F	TAGATTAGTATCTAAGTCTGTGG	5R	110464047-110464069	400
5R	TGTTATTTATAGACAACCCTCCA		110464424-110464446	
6F	ATCAGAATAGTGTGTGGAAGAG	6R	110465810-110465831	335
6R	AACACTATTTCCTCAGGCTAAC		110466123-110466144	
7F	CTTGAATGGTAATACTTGCTGAG	7F,8F,9R	110467233-110467255	1323
9R	GGACTCTTAGTTTTACCCAGAC		110468534-110468555	
8F	AATAGAAGTGAATGAATACTGGAG		110467774-110467867	
10F	TTTAATGAAGTAGTTGCAATCTGG	10F	110468743-110468766	405
10R	CTTATAGCAGAACCTCAATCAC		110469126-110469147	
11F	AGTGGTAATAACATCTTTGTTTTG	11R	110469508-110469531	379
11R	ACAAGACCAGCATGCACCTG		110469867-110469886	
12F	CTTGTTAGATTGGAAACATATTGC	12F	110470743-110470766	382
12R	AATATTATGATGAGAAACCTTGAG		110471101-110471124	
13F	TGAAGGCTAGTCCCATATATAG	13F,15R	110473485-110473506	1582
15R	ATTTGATCGCATCAACTCCCTG		110475045-110475066	
16F	TAAGGCAGCCTGAATGTTAGTC	16R	110476493-110476514	452
16R	GTTTCATGACACTACTACCTGC		110476923-110476944	
17F	GTGGTGACTTTCTGATCAATGC	17F	110482460-110482481	443
17R	AGCTGTCTACATTATCAAGCAG		110482881-110482902	
18F	GATGGCATCTATGACATAAGTC	18F,19R	110483926-110483947	945
19R	GCATTGTCAGTGCTGTCTTAC		110483850-110484870	
20F	CTGTGGTATTGGTCAGAAGAG	20F,21R	110487239-110487259	648
21R	ATCTTAACTACTGAGAACGCTG		110487866-110487866	
22F	TTGGTGTCATCGTTTGTACTG	22F,23R	110489013-110489033	1638
23R	GGAGGTGCGATTTTATTTCAAG		110490629-110490650	

Primers used to obtain the initial PCR amplicons and for subsequent sequencing of the WDR36 gene are listed in this table. The genomic positions of primers were retrieved from NCBI build 35.1.

bers of affected males and females. It was consistent with an autosomal dominant pattern of inheritance. Examination of the Mendelian inheritance of genotypes of all microsatellite markers showed that most markers in one male subject in the third generation had a Mendelian inheritance inconsistent with those in the other family members, indicating he was not a genetically related member of this family. Thus he and his son, together with his spouse, were excluded from our linkage analysis. To increase the statistical power, 23 informative meioses were used in our linkage analysis (Figure 1). Among them, there were a total of eight JOAG patients. For the purpose of linkage analysis, only JOAG patients were considered affected. The other family members were considered as normal or unaffected. Because the minimum and median ages at diagnosis were, respectively, 15 years (V:12) and 20 years old, this pedigree was considered a juvenile-onset, but not an adult-onset, open-angle glaucoma family.

Mutation screening of MYOC *and* OPTN*:* Since *MYOC* and *OPTN* are associated with autosomal dominant open angle glaucoma [14,21], we screened both genes for sequence alterations in all 27 family members whose blood samples were available. Three polymorphisms (R76K, -83G>A, and -1000G>C) in *MYOC* and four polymorphisms (T34T, M98K, R545Q, and IVS7+24G>A) in *OPTN* were found in this family. However, none of them segregated with JOAG and were thus not associated with glaucoma in the family [26].

Genome-wide scan: After exclusion of *MYOC* and *OPTN* as disease-causing genes in our family, a genome-wide search was carried out using the ABI MD-10 marker set. Microsatellite markers from this set are spaced on a 10 cM average grid on all 22 autosomes. Following a first scan, markers located in three regions on chromosomes 2, 5, and 7 demonstrated two-point LOD scores >1.0 (Table 2). Among these three regions, one marker at D5S436, on the long arm of chromosome 5 band q32, gave a LOD score value of 2.41 at θ=0.00, showing a suggestive linkage to JOAG. Two adjacent markers at loci D5S410 and D5S471 gave LOD score values of 1.48 and 1.05, respectively. This potential chromosome region on 5q23-q33 between D5S471 and D5S410 was thus further evaluated by fine mapping. The other two regions on chromosomes 2 and 7 that gave two-point LOD scores >1.0 (Table 2) were not further pursued in this study.

Fine mapping: In the flanking region of D5S471 and D5S410, 45 additional microsatellite markers were genotyped. Linkage analysis of most of these markers gave positive LOD score values (Table 3). A maximum two-point LOD score of 2.41 at θ=0.00 was obtained for D5S436. Markers D5S1505, D5S2098, and D5S402 also had LOD score values over 2.0.

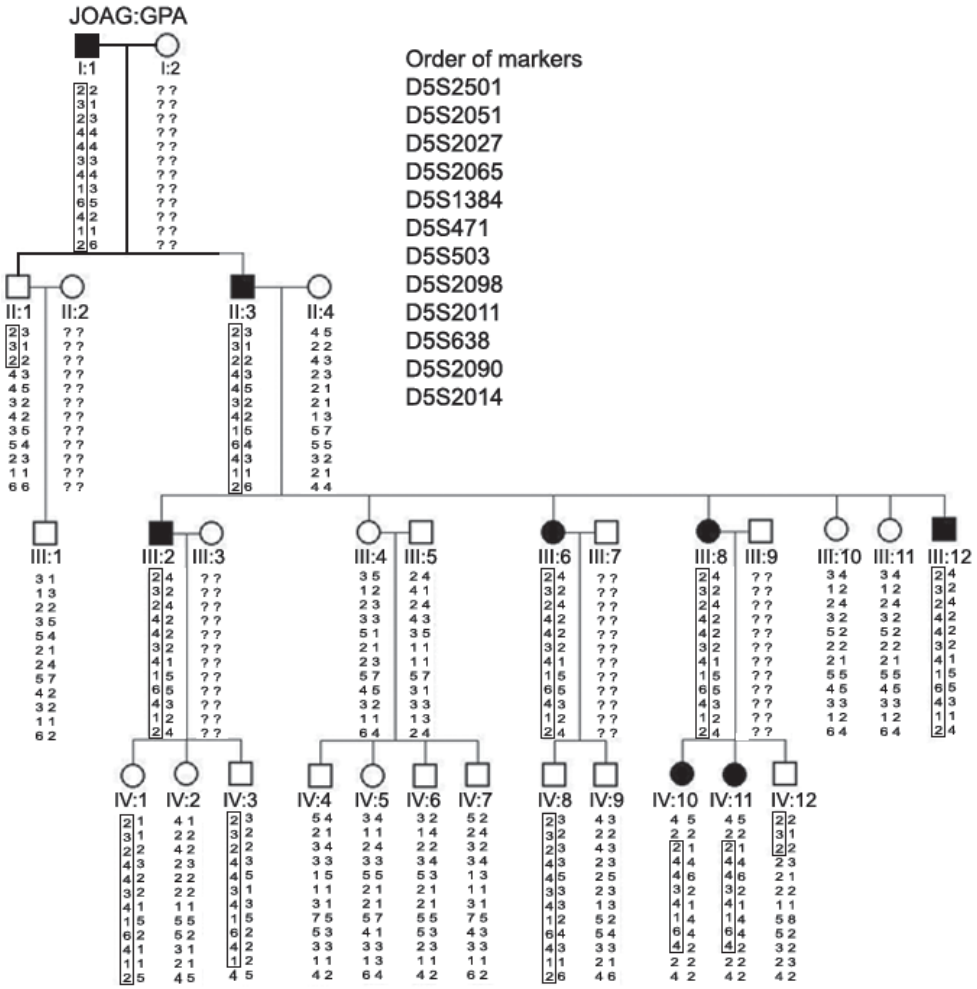

Figure 1. Haplotypes using markers from chromosome 5. Based on the previously published pedigree structure [26], only 30 informative family members were included in this figure. Square cells refer to males and circle cells refer to females. Shaded cells are JOAG. Segregating haplotypes are shown in the rectangles. The haplotype for subject I:1 was inferred by using known genotypes from other subjects in this family.

Multipoint analysis of these markers did not increase the LOD score values. When only affected meioses were used in two-point linkage analysis, a maximum LOD score of 1.81 at θ=0.00 were obtained for D5S2065, D5S1384, D5S2098, D5S2011, and D5S638. When the affected status of the unaffected family members was considered as normal for those with age greater than or equal to 35 years or as unknown for those with age <35 years in two-point linkage analysis, a maximum LOD score of 4.82 at θ=0.00 were obtained for D5S2011. Markers D5S2065, D5S1384, D5S471, D5S503, D5S2098, and D5S638 had LOD score values over 4.0 at θ=0.00. We used FASTLINK to generate 10,000 unlinked replicates with allele frequencies of D5S2011 and then computed the best LOD score for each replicate. No replicates exceeded the true LOD score of 4.82 and gave an empirical p<0.0001.

Haplotype analysis and recombination mapping: A total of 12 microsatellite markers within the flanking region of D5S471 and D5S410 were used to construct the haplotypes (Figure 1). At the time of study, inspection of the haplotype transmission data identified a common disease haplotype that has been clearly inherited by all seven affected subjects (II:3, III:2, III:6, III:8, III:12, IV:10, and IV:11) in this family, by the founder patient (I:1), and by three other at-risk subjects at 7 (IV:1), 3 (IV:3), and 13 (IV:8) years of age. These three phenotypically normal subjects carry 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 revealed two critical recombination events in two affected individuals (IV:10 and IV:11) that limited the location of the JOAG locus telomeric to D5S2051 and centromeric to D5S2090, within a region of about 36 Mb (Figure 1, Figure 2). This region was located on 5q22.1-q32.

Screening the WDR36 *gene at* GLC1G *for mutations:* Recently, *WDR36* was characterized as the third glaucoma gene [9]. The gene mapped at the *GLC1G* locus on chromosome 5q22.1. Even though *WDR36* localized centromerically to the present disease interval at chromosome 5q22.1-q32 between

Table 2. Markers with two-point LOD score values >1.0 in an initial genome-wide scan

		Two-point LOD score values at θ								
Marker	Location	0.00	0.01	0.05	0.10	0.20	0.30	0.40	Zmax	θmax
D2S2211	2p25	0.87	0.94	1.07	1.05	0.84	0.52	0.21	1.07	0.05
D5S471*	5q23	-inf	-1.81	0.09	0.72	1.05	0.93	0.57	1.05	0.20
D5S436*	5q32	2.41	2.37	2.21	2.00	1.56	1.09	0.57	2.41	0.00
D5S410*	5q33	-inf	0.57	1.27	1.48	1.44	1.11	0.61	1.48	0.10
D7S517	7p22	1.06	1.04	0.96	0.88	0.71	0.51	0.27	1.06	0.00

A total of 382 microsatellite markers with an average spacing of 10 cM were used in the initial genome-wide scan. The asterisk indicates the promising chromosome region on 5q23-q33 within a marker presented a suggestive LOD score value of Z_{max}=2.41 at θ=0.00.

Table 3. Fine mapping of chromosome 5q

			Two-point LOD score values at θ								
Marker	Marshfield map (cM)	Sequence map (Mb)	0.00	0.01	0.05	0.10	0.20	0.30	0.40	Zmax	θmax
D5S1466	117.51	109.0	-inf	-3.39	-1.32	-0.48	0.19	0.38	0.30	0.38	0.40
D5S2501	116.98	110.1	-inf	-7.52	-3.49	-1.90	-0.55	-0.02	0.13	0.13	0.40
D5S492	116.98	110.3	-inf	-4.20	-1.51	-0.47	0.33	0.52	0.39	0.52	0.30
D5S2051	116.98	111.0	-inf	-7.08	-3.55	-2.08	-0.79	-0.26	-0.10	-0.10	0.40
D5S2027	119.50		-inf	-7.05	-3.64	-2.25	-1.00	-0.40	-0.10	-0.10	0.40
D5S2065	122.01	113.7	-inf	-1.54	0.35	0.97	1.26	1.09	0.66	1.26	0.20
D5S1384	129.83	118.9	-inf	-1.54	0.34	0.97	1.26	1.09	0.66	1.26	0.20
D5S471	129.83	119.1	-inf	-1.81	0.09	0.72	1.05	0.93	0.57	1.05	0.20
D5S1505	129.83	119.2	2.11	2.08	1.95	1.79	1.43	1.02	0.55	2.11	0.00
D5S503	129.83	120.3	-inf	-1.67	0.21	0.84	1.15	1.00	0.61	1.15	0.20
D5S2098	131.48		-inf	1.60	2.06	2.07	1.78	1.33	0.74	2.07	0.10
D5S2497	137.39		-inf	0.44	1.04	1.19	1.11	0.85	0.45	1.19	0.10
D5S2011	144.06	141.3	-inf	-1.24	0.60	1.18	1.39	1.15	0.68	1.39	0.20
D5S402	147.49	144.4	2.11	2.08	1.95	1.79	1.43	1.02	0.55	2.11	0.00
D5S436	147.49	145.2	2.41	2.37	2.21	2.00	1.56	1.09	0.57	2.41	0.00
D5S638	148.63	146.7	-inf	-0.12	1.12	1.48	1.53	1.22	0.70	1.53	0.20
D5S2090	150.34	147.2	-inf	-5.78	-3.05	-1.93	-0.91	-0.41	-0.13	-0.13	0.40
D5S2014	153.17	149.9	-inf	-0.56	0.70	1.10	1.20	0.96	0.52	1.20	0.20
D5S410	156.47	152.8	-inf	0.57	1.27	1.48	1.44	1.11	0.61	1.48	0.10

A total of 45 additional microsatellite markers flanking the promising region on 5q23-q33 were analyzed. Only the 19 that flank the critical region on 5q22.1-q32 are shown in this table.

positions 111,037,156 bp and 147,210,429 bp using the Human Genome Sequence (Build 35.1), the discrepancy that currently exists between the genetic and physical maps may still position *WDR36* within our disease region, thus making the gene a good candidate for the disorder in our family. We therefore screened for mutations in all 23 coding exons and splicing junctions of the *WDR36* gene in three affected patients carrying the disease haplotype (subjects II:3, III:6, and III:8). Two asymptomatic persons, spouse III:3 and subject III:4, who did not harbor the disease haplotype, were used as controls. No sequence variations were detected in any of the three affected nor the two control persons in the 23 exons and splicing junctions. All sequences obtained were wild-type. Our data thus excluded coding sequences and splicing junctions of the *WDR36* gene as sites for potential mutations.

Candidate gene search: Within the 5q22.1-q32 region between D5S2051 and D5S2090, a total of 311 genes have been annotated in the NCBI Map Viewer (Build 35.1). Such a large number of genes pose technical difficulties for candidate gene search by mutation analysis. It will be helpful to further confine the interval by recruiting more family members to identify additional recombinational events.

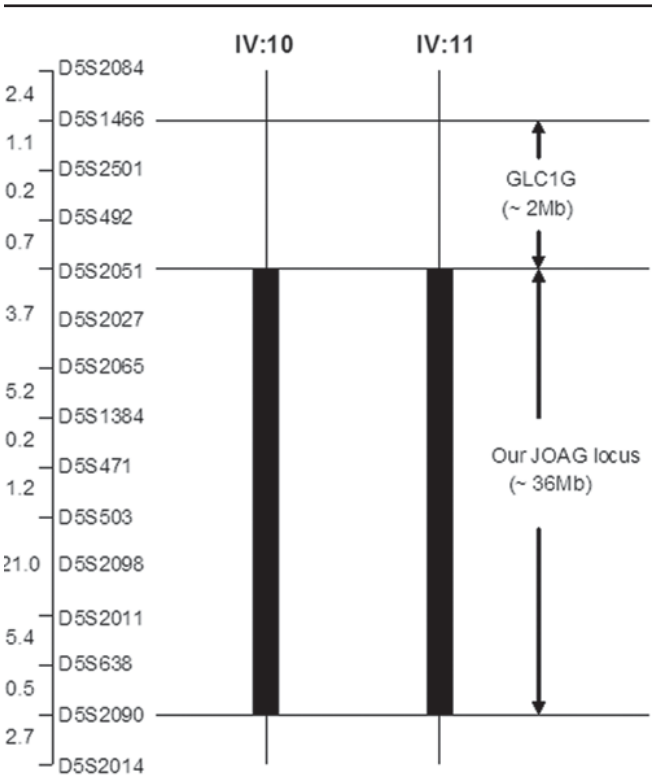

Figure 2. Recombination mapping of chromosome 5. Solid rectangles indicate the nonrecombinant region for each individual. The critical recombination events are shown by horizontal lines. The JOAG locus on chromosome 5 is confined centromerically at D5S2051 and telomerically at D5S2090, within a region of 36 Mb. The JOAG locus identified in our study was very close to but did not overlap with the *GLC1G* minimal interval between D5S1466 and D5S2051.

DISCUSSION

To date at least 15 genetic loci have been linked to POAG. Among those, there is evidence that JOAG can be caused by multiple independent genes located at heterogeneous loci [12]. The mapping of the first locus for JOAG was followed by the localization of several loci predicted to be involved in this severe type of glaucoma [3]. Two additional loci have been recently described [12]. In this study, we provide evidence for a novel locus that should harbor a gene responsible for JOAG, excluding *MYOC*, *OPTN*, and *WDR36*. We detected significant linkage with DNA markers at 5q23-q33. Fine mapping with additional markers within this region supported the linkage. Haplotype analysis and recombination mapping further confined this region to 5q22.1-q32. Monemi and colleagues [32] reported a probable adult-onset POAG locus on 5q33-q35, a region of approximately 36 cM between D5S2497 and D5S498. They termed this locus *GLC1G* and claimed that it had been narrowed down to an interval of <5 cM by using additional small families, from which one potential candidate gene was proposed [33]. More recently, the same group further refined their mapping interval, and new data resulting from discrepancies between the genetic and physical maps positioned several families previously mapped to the 5q33-q35 region more centromeric to chromosome 5q22.1. Saturation mapping of these linked families was subsequently performed. Meanwhile, Samples et al. [34] mapped a new POAG locus to a 3.8 Mb region between D5S2084 and D5S492 on chromosome 5 in a large Oregon family. By combining data obtained from Samples et al. [34], Monemi et al. [9] finally identified the third POAG gene as WDR36 at GLC1G on 5q22.1.

The JOAG locus identified in our study was very close to but did not overlap with the *GLC1G* minimal interval between D5S1466 and D5S2051 (Figure 2). However, discrepancy between the genetic and physical maps might still position the *WDR36* gene within our disease interval. We therefore screened *WDR36* for mutations and did not find any sequence variations in the coding exons or splicing junctions of the *WDR36* gene. Although we cannot rule out other variations outside these regions (within the introns or the promoter sequence), our data do suggest the presence of a novel JOAG gene on 5q. In addition, our haplotype analysis and recombination mapping excluded the interval between D5S2084 and D5S492 from being the disease-causing locus in our pedigree. This whole region had been reported by Samples et al. [34]. Since the 5q22.1-q32 region covers a large distance of about 36 Mb, recruitment of more family members and further mapping should help to refine this region. We conclude that a novel genetic locus on 5q22.1-q32 has been identified for juvenile-onset POAG.

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