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Localization of the Fourth Locus (GLC1E) For Adult-Onset Primary Open-Angle Glaucoma to the 10p15-p14 Region

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Summary

One of the major causes of blindness is primary open-angle glaucoma, which affects millions of elderly people worldwide. Genetic studies have so far mapped three loci for the adult-onset form of this condition to the 2cen-q13, 3q21-q24, and 8q23 regions. Herein, we report the localization of a fourth locus, to the 10p15-p14 region, in one large British family with a classical form of normal-tension open-angle glaucoma. Of the 42 meioses genotyped in this pedigree, 39 subjects (16 affected) inherited a haplotype compatible with their prior clinical designation, whereas the remaining 3 were classified as unknown. Although a maximum LOD score of 10.00 at a recombination fraction of $\theta = .00$ was obtained with D10S1216, 21 other markers provided significant values, varying between 3.77 and 9.70. When only the affected meioses of this kindred were analyzed, LOD scores remained statistically significant, ranging from 3.16 (D10S527) to 3.57 (D10S506). Two critical recombinational events in the affected subjects positioned this new locus to a region of ~21 cM, flanked by D10S1729 and D10S1664. However, an additional recombination in a 59-year-old unaffected female suggests that this locus resides between D10S585 (or D10S1172) and D10S1664, within a genetic distance of 5–11 cM. However, the latter minimum region must be taken cautiously, because the incomplete penetrance has previously been documented for this group of eye conditions. A partial list of genes that positionally are considered as candidates includes NET1, PRKCT, ITIH2, IL2RA, IL15RA, IT1H2, hGATA3, the mRNA for open reading frame KIAA0019, and the gene for D123 protein.

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Introduction

Glaucomas are a group of eye disorders with diverse clinical manifestations and a variable age at onset, from birth or early childhood to very late in life (Shields et al. 1996). They are one of the three most common causes of blindness worldwide (Thylefors and Negrel 1994; Quigley 1996; Wilson and Matrone 1996). Although the primary congenital and juvenile types of glaucoma are relatively uncommon, the insidious adult type, primary chronic open-angle glaucoma (COAG), accounts for the majority (70%) of glaucoma cases in mainly white populations, and its prevalence has been found to be four or more times higher in those of mainly African extraction (Leske 1983; Quigley and Vitale 1997). However, in Asian and, especially, in Inuit populations, primary angle-closure glaucoma, which has a different pathogenesis, may have an equal prevalence or may predominate. Open-angle glaucoma accounts for ~3% of the blindness in white and 7.9% in black American populations (Tielsch et al. 1991; Quigley 1996; Quigley and Vitale 1997). This condition is clinically diagnosed by three tests to reveal characteristic glaucomatous optic nerve damage; characteristic visual field loss; and increased intraocular pressures (IOP) (Crick 1974; Quigley 1993; Wilson and Matrone 1997). Although IOP is not solely accountable for this phenotype, it is, nevertheless, a major contributory factor. Normal- or low-tension glaucoma (NTG or LTG) is a form of open-angle glaucoma in which typical glaucomatous cupping of the optic nerve head (ONH) and visual field loss are present but in which the recorded IOPs are consistently within the statistically normal range of $\leqslant 22$ mmHg (Hitchings 1992; Grosskreutz and Netland 1994; Werner 1996). NTG is now believed to be more frequent than was originally thought, accounting for about one-fifth of primary open-angle glaucoma (POAG), although a single screening test may record NTG in more than one-half of the cases (Tielsch et al. 1991; Werner 1996). Most COAG patients do not manifest the disease before the age of 40 years, even though the first sign of the condition may have appeared earlier but was not detected.

This is probably because there were no symptoms to make the patients aware that a test was necessary or the condition was not detected even when examined routinely by the usual methods, which are sometimes inadequately carried out. Earlier changes can now be detected in several ways. Using confocal laser scanning systems (e.g., the Heidelberg retinal tomograph), the topography of the ONH (Weinreb et al. 1989; Dreher et al. 1991) and the thickness of the nerve fiber layer (Béchtoille et al. 1997) can be better assessed. Early changes in visual fields can be detected by more accurate methods of measurements of progression (Fitzke et al. 1996; Crabb et al. 1997). In addition, newer indicators, such as motion detection (Baez et al. 1995) and contrast sensitivity (Yu et al. 1991), are being evaluated, as are blue/yellow perimetry (Johnson et al. 1993) and pattern electroretinography techniques (Korth et al. 1993).

The late onset and painless nature of this condition have serious implications for diagnosis and proper treatment of COAG. As a consequence, it has been difficult to determine the exact mode of inheritance for this condition, since the majority of the patients are either isolated cases or, by the time of first presentation, no accurate clinical information or systematic ophthalmic examination would be available on previous generations. Therefore, autosomal dominant, autosomal recessive, X-linked, and even multifactorial modes of inheritance have been suggested (Francois 1966; Jay and Peterson 1970; Netland et al. 1993; Lichter 1994; Johnson et al 1996). However, in the majority of COAG families that have been systematically studied, the autosomal dominant mode of inheritance with a reduced penetrance has been suggested (Avramopolus et al. 1996; Richards et al. 1996; Stoilova et al. 1996; Wirtz et al. 1997; Trifan et al., in press). A number of reports described families with both NTG and high-tension glaucoma subjects within the same family (Stoilova et al. 1996; Werner 1996). Conversely, few simple NTG families are documented in which the phenotype is transmitted as an autosomal dominant trait (Bennett et al. 1989).

The genetic linkage study of POAG families has been the subject of a number of reports in the literature. Thus far, a locus for the juvenile open-angle glaucoma (i.e., GLC1A; MIM 137750) has been assigned to the long arm of chromosome 1 (Sheffield et al. 1993; also see Raymond 1997; Sarfarazi 1997), and more recently a number of mutations in the trabecular meshwork–induced glucocorticoid-response protein (TIGR) have been reported in different families (Adam et al. 1997; Stoilova et al. 1997; Stone et al. 1997). For COAG, the first locus (GLC1B; MIM 137760) has been mapped to 2cen-q13 (Stoilova et al. 1996) and the second locus (GLC1C; MIM 601682) to 3q21-q24 (Wirtz et al. 1997), and, more recently, we mapped the third locus (GLC1D) to the 8q23 region (Trifan et al., in press). In this article, we present the chromosomal localization of a new locus (i.e., GLC1E) to the 10p15-p14 region in one large family with a typical normal-tension POAG.

Material and Methods

Family Ascertaiment

A single large family (POAG-90) has been ascertained through the database of the International Glaucoma Association, London. Consent to contact other family members for their participation in the study was obtained from the proband. The study protocol and consent forms were approved by the University of Connecticut Health Center Institutional Review Board. For the clinical reports, we have depended, to a considerable extent, on cooperating consultant colleagues, in view of the wide geographic spread of the family members. Family relationships of glaucoma subjects and their normal kinship have also been obtained. Clinical information included, but was not limited to, data on the age at onset/diagnosis, IOP before and after treatment, stages of visual field loss, evidence for glaucomatous optic-nerve change, cup/disc ratios, past eye surgery, current eye medication, and type and primary/secondary nature of glaucoma, as well as other associated clinical findings for each individual member of the pedigree. A summary of clinical information obtained on the affected members of this family is given in table 1. All affected members had POAG with no associated abnormalities and were diagnosed in early to late adulthood. The clinical diagnosis of both affected and normal members of this family has been assigned by their respective ophthalmologists. Affected status was affirmed after ophthalmologic examination, which normally included tonometry, gonioscopy, slit-lamp examination, and visual field tests. The diagnosis was based on the characteristic appearance of the optic disc, typical visual field loss, and normal open anterior chamber angle. IOPs in the majority of the affected members of this family were measured, as always, at <22 mmHg, and, therefore, this kindred was classified as a typical NTG family. As shown in figure 1, the glaucoma phenotype in this pedigree segregated as an autosomal dominant condition with no documented skipped generation. A total of 46 individuals consented to participate in this study and to donate specimens (blood, buccal cells from mouth washes, and skin biopsies), including samples from 15 affected subjects. DNA samples from six other at-risk subjects were not available at the time of this study. The genotype of one additional affected subject (II:1) was inferred from his offspring and his spouse's genotypes. Because no clinical information was available for one deceased subject

Table 1

Clinical Data on 15 Affected Subjects in the POAG-90 Family

Subject	Sex	Age at Diagnosis (years)	Highest IOP Recorded		ONH		Cup/Disk Ratio		Visual Fields		Treatment
			OD	OS	OD	OS	OD	OS	OD	OS	
II:3	M	45	24	23	A	A	.90	.90	A	A	Timolol maleate
III:1	F	40	15	15	A	A	.90	.90	A	A	Timolol maleate, trabeculectomy OS
III:5	M	49	17	20	N	A	.70	.70	N	A	Nil
III:6	F	38	22	23	A	A	.80	.90	A	A	Betaxolol hydrochloride, trabeculectomy
III:8	M	58	16	15	A	A	.70	.80	A	A	Timolol maleate
III:10	M	35	14	20	A	A	.80	.80	A	A	Surgery (OD)
III:11	F	62	25	21	A	N	.80	.50	A	A	Timolol maleate
III:14	F	40	16	16	A	A	.95	.95	A	A	Timolol maleate
III:17	F	52	19	17	A	A	.85	.85	A	A	Timolol maleate
III:19	M	36	20	20	A	A	.70	.70	A	A	Timolol maleate
III:20	F	65	18	19	A	A	.80	.80	S	A	Nil
IV:1	F	28	19	19	N	A	.60	.70	A	A	Nil
IV:2	F	23	18	18	A	A	.80	.85	A	A	Timolol maleate
IV:11	F	47	16	16	A	A	.90	.90	A	A	Betaxolol hydrochloride
IV:20	F	40	21	18	A	A	.60	.60	A	A	Nil

NOTE.—OD = right eye; OS = left eye; A = abnormal changes of a glaucomatous type; S = suspicious of glaucomatous change; and N = normal.

(II:9), her clinical status remained unknown. This pedigree consisted of 51 segregated meioses, of which 18 (11 female and 7 male) were affected with POAG. The vertical inheritance of the POAG phenotype, together with a direct male-to-male transmission, is consistent with an autosomal dominant pattern of inheritance in this pedigree.

Molecular Analysis

DNA was extracted from peripheral blood (Moore 1994), buccal cells (DNAzol, Molecular Research Center), or skin fibroblasts (Trizol, Gibco BRL). Genomic DNA was amplified by PCR using sequence-specific primers. Most of the amplifications were carried out by multiplex PCR of two to three short-tandem-repeat polymorphism (STRP) markers. Information on primer sequences, number of alleles, band sizes, type of polymorphisms, and order of markers were obtained from the Genome Data Base (Fasman et al. 1994), Généthon (Dib et al. 1996), the Utah Marker Development Group (1995), the Cooperative Human Linkage Center (Sheffield et al. 1995), the Chromosome 10 web site (at Genome Therapeutics Cooperation [http://www.cric.com/htdocs/sequences/chr10-mapping/index.html]), and the Marshfield Center for Medical Genetics. Additional information on the radiation hybrid and YAC contig map were obtained from the Whitehead (Hudson et al. 1995) and Stanford Institutes for Genome Research. The oligonucleotide primers used in this study were purchased from Research Genetics. Amplification conditions varied for different markers, but usually a 2-min initial denaturation at 94°C was followed by 30–35 cycles of denaturation at 94°C for 30 s and annealing at 55°C–60°C for 40 s (depending on specific primers), with a final extension at 72°C for 2 min. We used 25 μl total volume per reaction consisting of 1 × PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1–1.5 mM $MgCl_2$), 0.25 mM each primer, 100 ng template DNA, 50 mM each dNTP, and 0.25 U DNA polymerase (AmpliTaq, Perkin-Elmer). The PCR reaction was carried out on a GeneAmp 9600 PCR system (Perkin-Elmer). The amplified products were separated by electrophoresis on a 6%–7% denaturing polyacrylamide gel. The gels were subsequently silver stained (Bassam et al. 1991), photographed, and thereafter manually genotyped for the STRP markers.

Linkage Analysis

The obtained genotypic results were entered into a dedicated computer program (Database Management System; author's unpublished program) for Mendelian error checking and data inconsistencies and were subsequently used to prepare genotypic data for analysis by the LINKAGE program (Lathrop and Lalouel 1984). Two-point linkage was calculated using the MLINK component of the LINKAGE package (FASTLINK version 3.0P; Schaffer et al. 1994). LOD scores were obtained under the assumption of an autosomal dominant disorder with a frequency of .0001, no phenocopy, and complete penetrance, since no skipped generations were observed in this family. Multipoint linkage analysis was

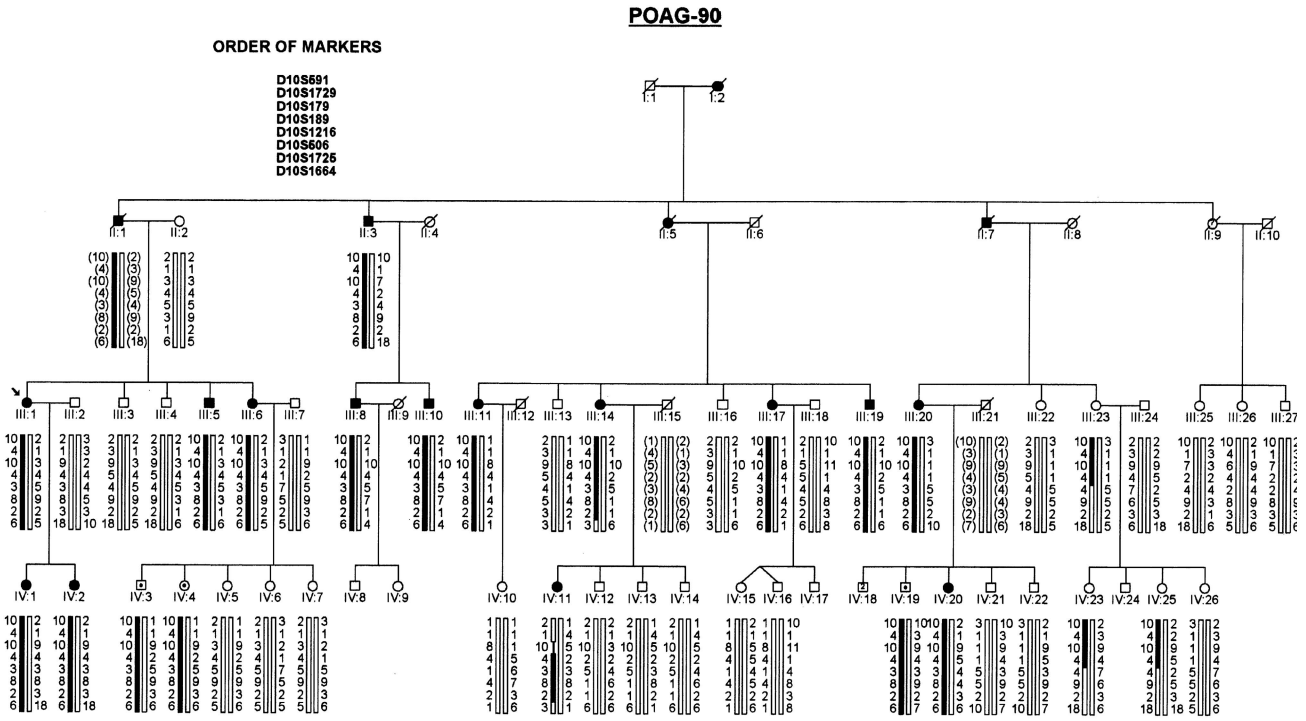

Figure 1 Pedigree structure of the POAG-90 family, segregating for NTG. The order for a selected group of STRP markers presented in this kindred is given at the top left corner of the figure. Inherited haplotypes are shown below each subject. The solid bars indicate the affected-bearing haplotypes, and the open bars indicate the normal haplotypes. The uninformative portion of haplotype in individual IV:11 is shown with a thin black bar in the middle. Note that individual III:14 is recombinant for the centromeric marker D10S1664, whereas her daughter (IV:11) is recombinant for the telomeric marker D10S1729. The three gene carriers who have inherited the affected-bearing haplotype are shown with a dot inside their symbols (IV:3, IV:4, and IV: 19). The proband (III:1) is indicated with an arrow.

obtained using the LINKMAP module of the LINKAGE program. Because of the reported late onset in this group of eye conditions and the lack of any reliable penetrance ratios, and in order to eliminate the effect of possible incomplete penetrance, the LOD score calculations were repeated for the affected meioses only. Haplotypes for the entire set of markers were constructed and evaluated for genotype inconsistency and inheritance from both parental generations.

Results

Clinical Phenotype

Clinical findings in the glaucoma patients from family POAG-90 are described in detail in table 1. Of the 15 affected subjects presented in this table, 11 had all the three common diagnostic criteria of abnormal ONH, large cup/disc ratios, and visual field loss in both eyes. For the remaining four affected subjects, one individual (III:5) was definitely affected in his left eye but in his right eye had a cup/disc ratio of 0.7, but both visual fields and ONH were normal. This 51-year-old subject, on his last visit, had an inferior scotoma in the left eye, and the right eye was normal, when checked by the Humphrey 24-2 visual field analyzer. His optic disc also showed vertical cupping of 0.7 in both eyes, and his IOP measurements were 20 mmHg in the left eye and 17 mmHg in the right eye (table 1). When his optic discs were measured on the scanning laser ophthalmoscope, his left disc was abnormal superiorly, which concurred with the inferior field defect, but his right disc was normal. Although this subject is definitely affected in his left eye, his right eye needs to be evaluated on a regular basis. Another subject (III:11) was affected with typical glaucomatous changes in her right eye and normal ONH but abnormal visual field changes in her left eye. Individual III:20 had abnormal optic discs, with a cup/disc ratio of 0.8 in both eyes, and an abnormal upper arcuate loss of field in the left eye, with suspicion of arcuate depression of the right field, and so she was classified as a left NTG with only a suspicion of the same condition in the right eye. Finally, the last individual (IV:1) was affected with typical glaucomatous changes in her left eye and abnormal visual field, with cup/disc ratio of 0.6 in her right eye. Therefore, since the questionable eye in these subjects either had abnormal ONH, abnormal vi-

sual field loss, or large cup/disc ratio and, furthermore, since the other eye was definitely affected, the clinical status of these four subjects was coded as affected. Although NTG is usually a bilateral condition, one eye often becomes affected ahead of the other eye, so the assignment of affected status to the four subjects discussed above is considered justifiable, since one of their eyes was definitely affected with glaucoma. Figure 2 shows the visual fields for the proband (III:1) in family POAG-90. In the left eye, severe upper arcuate loss and marked lower arcuate loss are apparent, whereas, in the right eye, marked upper and lower arcuate loss are evident. Figure 3 shows both the optic discs and visual fields for the proband's youngest daughter (IV:2). Both optic discs are grossly abnormal when compared with an age-match group, the left disc being worse than the right. The right visual field shows a nasal step with slight upper and lower arcuate depression of sensitivity, which is compatible with glaucoma (fig. 3*A*), whereas the left field shows a ring-type scotoma with marked upper and lower arcuate loss, which is of a glaucomatous type (fig. 3*B*).

The age at diagnosis in the family was documented to vary between 23 and 65 years, with a mean of 44 years. The IOPs in the affected family members varied, but they were generally in the normal range. Twelve of 15 affected members had IOPs consistently <22 mmHg. The remaining three patients have had IOPs of 23, 24, and 25 mmHg, recorded at some time during their follow-up visits. The phenotype consisted of a slow progression that was accompanied by glaucomatous optic nerve damage, large cup/disc ratios, and an eventual visual field loss. Five affected and three normal subjects had myopia. Of 15 affected family members (5 male and 10 female) for whom a detailed nonocular clinical history was available, 3 females gave a history of occasional present or past episodes of migraine, and 1 female gave a definite history of Raynaud phenomenon. Therefore, this is not a family in which vascular mechanisms increase optic nerve vulnerability to a relatively low rise in IOP. There was no history of hypertension or diabetes in those affected. In summary, we did not find any particular relationship between the myopia, systemic abnormalities, and glaucoma in this family.

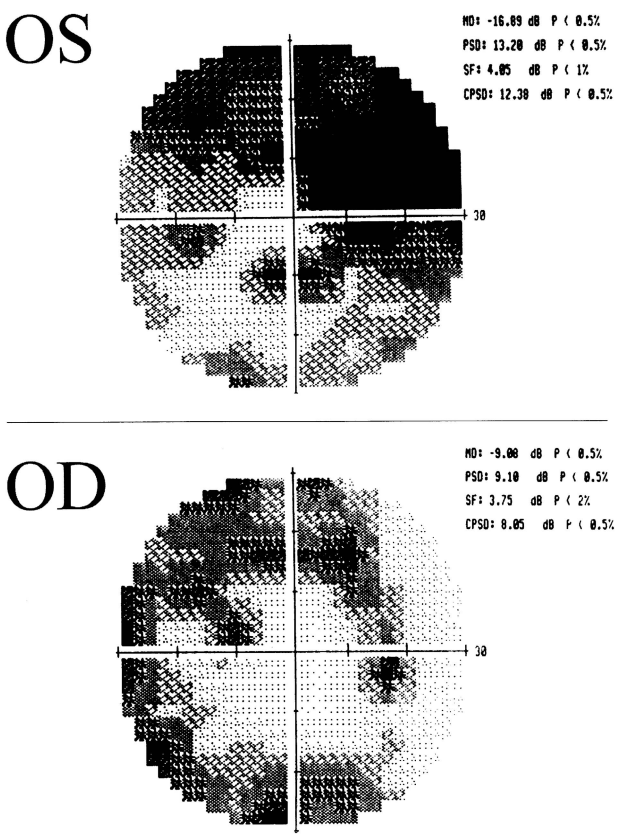

Figure 2 Visual fields (Humphrey visual field analyzer) for the proband (III:1) in pedigree POAG-90. In the field for the left eye (OS; *top*), severe upper arcuate loss and marked lower arcuate loss are apparent. For the right eye (OD; *bottom*), both marked upper and lower arcuate loss are seen. Both visual fields are typical of POAG. Global indexes are as follows: MD = mean deviation; PSD = pattern SD; SF = short-term fluctuation; CPSD = corrected pattern SD, all for which probabilities (%) are given.

Genetic Linkage Study

After an initial exclusion of linkage to selected DNA markers from the sites of the GLC1A on 1q, GLC1B on 2q, GLC1C on 3q, and GLC1D on 8q, a series of candidate gene loci that could play a role in the etiology of glaucoma in this family were also screened. However, linkage study of these DNA markers did not reveal any indication of linkage. Furthermore, in view of a number of mutations reported in the TIGR gene (MIM 601652) for both juvenile- and adult-onset types of POAG, we screened the proband of this family with this gene. SSCP of the coding regions of the TIGR gene did not reveal any mutations in this family. Subsequent to a random genomewide search, a hint of linkage was obtained with one of the DNA markers located on the short arm of chromosome 10, band p15-p14 (i.e., D10S1172). An additional 23 markers from the same region were used to saturate the area surrounding this newly identified location (designated the "GLC1E" locus).

A total of 22 DNA markers located within the 10p15-p14 region provided LOD scores of >3.0 in this family (table 2). Construction and inspection of the haplotype transmission data (fig. 1) also showed a common affected haplotype that has been clearly inherited by a total of 15 living and 1 deceased (whose genotypes were inferred from his 5 living offspring and spouse) affected subjects and 3 other at-risk subjects who were 19

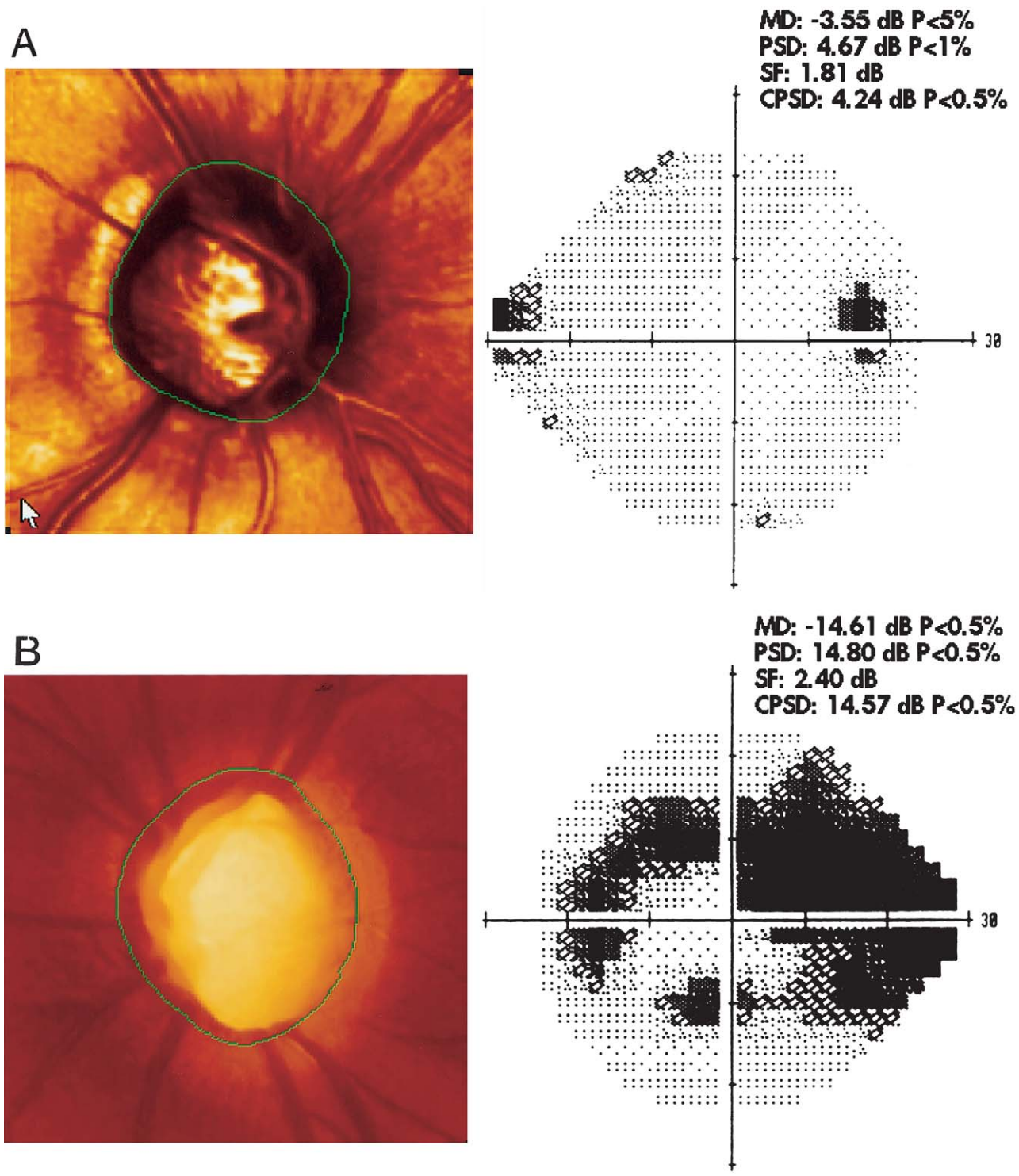

Figure 3 Optic discs (Heidelberg retinal tomograph) and visual fields (Humphrey visual field analyzer) for the proband's youngest daughter (IV:2). *A,* Right visual field, showing nasal step with slight upper and lower arcuate depression of sensitivity. *B,* Left visual field, with marked upper and lower arcuate loss resulting in a ring scotoma. As in figure 2, the global indexes and their probabilities are given. Both optic discs are grossly abnormal when compared with an age-match group, with the left disc (*B*) being more affected than the right (*A*). The findings are typical of POAG, which is much more marked in the left eye.

Table 2

LOD Score between the GLC1E locus and 24 DNA Markers on the 10p15-p14 Region

	LOD Score at θ =										All Meioses		Affected Only	
Marker	.00	.05	.10	.15	.20	.25	.30	.35	.40	.45	Z_{max}	θ_{max}	Z_{max}	θ_{max}
D10S1153	−∞	6.66	6.47	5.98	5.35	4.61	3.78	2.87	1.88	.85	6.66	.06	2.14	.06
ATA84DO2	−∞	4.58	4.55	4.24	3.79	3.25	2.62	1.93	1.19	.47	4.62	.07	2.85	.00
D10S591	−∞	5.52	5.43	5.05	4.54	3.92	3.22	2.45	1.61	.73	5.54	.06	.79	.10
D10S1729	−∞	5.52	5.20	4.73	4.17	3.55	2.88	2.14	1.36	.57	5.55	.04	1.81	.00
D10S1713	−∞	5.22	4.91	4.44	3.90	3.30	2.66	1.97	1.26	.54	5.25	.04	2.70	.00
D10S179	−∞	6.61	6.21	5.65	4.99	4.25	3.45	2.58	1.66	.72	6.66	.03	3.32	.00
D10189	−∞	4.45	4.29	3.95	3.50	2.98	2.39	1.75	1.06	.39	4.45	.05	1.97	.00
D10S1751	6.09	5.67	5.18	4.65	4.06	3.43	2.75	2.04	1.29	.54	6.09	.00	2.91	.00
D10S1691	5.25	5.06	4.70	4.27	3.76	3.21	2.60	1.95	1.25	.54	5.25	.00	2.01	.00
D10S1779	4.35	4.40	4.17	3.82	3.39	2.90	2.36	1.77	1.13	.47	4.44	.03	2.93	.00
D10S1172	8.32	7.87	7.24	6.53	5.74	4.89	3.97	2.98	1.94	.87	8.32	.00	3.32	.00
D10S1431	2.72	2.47	2.22	1.95	1.68	1.40	1.12	.83	.54	.27	2.72	.00	1.33	.00
D10S1420	5.89	5.41	4.91	4.38	3.81	3.22	2.59	1.94	1.26	.59	5.89	.00	2.08	.00
D10S1412	4.63	4.23	3.81	3.37	2.92	2.45	1.96	1.46	.95	.46	4.63	.00	1.77	.00
D10S585	4.24	3.91	3.55	3.16	2.75	2.31	1.85	1.36	.84	.34	4.24	.00	2.48	.00
D10S1216	10.00	9.20	8.36	7.47	6.54	5.55	4.50	3.39	2.22	1.01	10.00	.00	3.43	.00
D10S2325	7.85	7.30	6.67	5.97	5.22	4.41	3.54	2.61	1.62	.63	7.85	.00	2.78	.00
D10S1430	3.77	3.48	3.18	2.86	2.51	2.15	1.76	1.36	.93	.47	3.77	.00	2.05	.00
D10S527	7.07	6.57	5.98	5.32	4.62	3.87	3.08	2.26	1.41	.59	7.07	.00	3.16	.00
D10S506	9.70	8.91	8.09	7.22	6.30	5.34	4.32	3.25	2.12	.94	9.70	.00	3.57	.00
D10S1725	2.31	2.09	1.87	1.64	1.39	1.14	.88	.61	.35	.13	2.31	.00	.84	.00
D10S1664	−∞	6.00	5.64	5.11	4.49	3.80	3.05	2.24	1.38	.54	6.04	.03	2.54	.06
D10S191	−∞	5.43	5.40	5.05	4.55	3.95	3.25	2.47	1.62	.74	5.48	.07	2.44	.06
D10S674	−∞	3.04	3.12	2.94	2.64	2.27	1.84	1.37	.87	.40	3.13	.08	1.21	.10

(IV:3), 17 (IV:4), and 41 (IV: 19) years old at the time of this study. Since these three normal subjects are still below the mean age at onset in this family (i.e., 44 years), they are still at risk for developing this condition in the future and therefore are considered to be "gene carriers" for this phenotype. Furthermore, we have identified 20 unaffected subjects who have inherited an entire normal chromosome from their affected parents. One normal subject (III:23), who is now 59 years old, was recombinant for the telomeric portion of the affected-bearing chromosome and has passed this to her two normal daughters (IV:23 and IV:25). However, these three subjects shared a minimum normal-bearing region of a haplotype that is not recombined with the glaucoma phenotype. This minimum region includes marker D10S1216, which incidentally provided the maximum linkage information data for this family. In summary, of a minimum of 42 directly scorable meioses in this family (including II:1, who was inferred), a total of 39 subjects inherited either part of or the entire chromosome that is complementary to their prior clinical classification. The other three affected-haplotype–carrying normal subjects are still too young to show any sign of this condition and, therefore, may develop glaucoma at some time in the future or, alternatively, may remain as asymptomatic gene carriers.

For the purpose of statistical linkage evaluation by the classical parametric method of LOD score analysis, the status of the three subjects mentioned above with affected-bearing haplotypes was coded as unknown, whereas the clinical status of the three normal subjects (III:23, IV:23, and IV:25), who were recombinants for a portion of the same affected-bearing haplotype, was coded as normal. The two-point linkage between the disease locus and DNA markers provided a maximum LOD score of $Z_{max} = 10.00$ at a recombination fraction of $\theta = .00$ with DNA marker D10S1216. Table 2 shows the LOD scores with a group of other STRP markers from the same region of this chromosome. As shown in this table, there is an overwhelming evidence for linkage of this family (i.e., LOD values of 3.77–10.00) to a total of 22 STRP markers from the 10pl5-p14 region. We have further examined the genetic linkage relationship of this family to the same DNA markers for only the affected subjects of this pedigree. Although this has resulted in reduction of LOD scores, as expected, the scores have remained statistically significant (i.e., $Z_{max} > 3.0$). For example, we have obtained LOD scores of 3.32 (D10S179 and D10S1172), 3.43 (D10S1216), 3.16 (D10S527), and 3.57 (D10S506) at $\theta = .00$. A four-point linkage analysis between the disease phenotype and three DNA markers of D10S591, D10S1216, and D10S1664 produced Z_{max} values of 10.17 at $\theta = .00$ for the entire family and a corresponding Z_{max} value of 3.72

when only the affected meioses were used. The minimal increase in the LOD score obtained with this multipoint analysis can be expected, since the D10S1216 marker was fully informative in this family and, when used alone, provided corresponding Z_{max} values of 10.00 (when the entire family was used) and 3.43 (when only the affected meioses were used).

The pedigree size and availability of genotypic data for a total of 26 unaffected subjects in this family prompted us to evaluate the potential influence of incomplete penetrance on the overall values of the LOD score. In order to do this, three liability classes were defined for the glaucoma phenotype in this pedigree. They consisted of class I, for all spouses who married into the pedigree, class II, for all the affected individuals, and class III, for the normal and healthy offspring who were born to an affected parent and were therefore at risk for developing the condition. We evaluated the LOD scores for the most informative marker, D10S1216, assuming an incomplete-penetrance rate that varied between 0% and 50%, once by coding the three "gene carriers" mentioned above as unknown and, next, by repeating the LOD score calculation when they were coded as "normal" and "at risk." All other normal offspring were also coded as being "at risk." As shown in figure 4, when these three subjects were coded as gene carriers, sequentially decreasing LOD scores of 10.00–7.01 were obtained for different values of incomplete-penetrance rates (fig. 4, curve A). However, when these three subjects, like other unaffected offspring of this pedigree, were coded as at risk (with full penetrance, a Z_{max} value of 6.31 at $\theta = .08$ was obtained), a Z_{max} value of 7.26 was obtained at an incomplete-penetrance rate of 18% (i.e., complete penetrance rate of 82%). Other LOD scores varied between 6.58 at 5% rate and 6.48 at 50% rate (fig. 4, curve B). Therefore, this result suggested that the penetrance rate for this pedigree and possibly for the POAG group of eye conditions, may be ~82%. This obviously will have an implication for those involved in the gene mapping of this group of eye conditions, because the number of potential unaffected gene carriers in their pedigrees may be $\leqslant$18%, thus reducing the power of linkage detection in the families being studied.

Inspection of haplotypes in this pedigree revealed two critical recombinants in two affected individuals that limited the location of the GLC1E locus telomerically to D10S1729 and centromerically to D10S1664, within a genetic distance of ~21 cM (figs. 1 and 5). However, because of the lack of informativeness of a number of DNA markers, the precious site of recombination (see fig. 5) for a region of ~4 cM that is covered by D10S552 and D10S189 cannot be determined. Therefore, the critical region of interest is likely to be within a 17–21 cM region that is covered by the latter two markers. Furthermore, we have observed another crossover in an unaffected 59-year-old female (III:23) who has inherited the telomeric portion of the affected-bearing chromosome, including D10S1172 (fig. 5). However, the extent of this recombination for other markers mapping between this marker and D10S585 cannot be determined. Thereafter, for all the centromeric markers located between D10S585 and D10S674, she has inherited the normal-bearing portion of the chromosome (figs. 1 and 5). If neither she nor any of her offspring is ever going to develop glaucoma, it is likely that the GLC1E locus could be limited to a region that is flanked by D10S585 and D10S1664, within a region of ~5 cM. However, because the incomplete penetrance has been well documented for this group of ocular conditions, one must consider the latter confined region cautiously. There are at least three YAC clones from this region that have been physically mapped (fig. 5). Although clone 809-F-9 has been mapped to 10p15, the other two YAC clones, 747-H-8 and 808-A-2, have been mapped to the 10p14 band (see fig. 5). Therefore, the physical location of the GLC1E locus is also on the 10p15-p14 region. However, if one takes into account the recombination event in the above-mentioned unaffected subject, the GLC1E locus would be expected to reside on band p14 of chromosome 10.

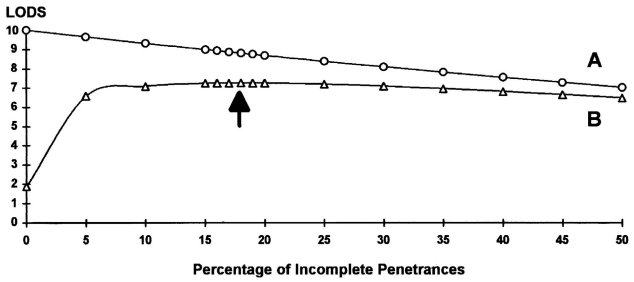

Figure 4 Effect of incomplete-penetrance rates on LOD scores of D10S1216 at $\theta = .00$, when the three gene carriers were coded as (*A*) unknown or (*B*) at risk. For the latter, the arrow points to a Z_{max} value of 7.260 at an incomplete-penetrance rate of 18%.

Discussion

In recent years, researchers have been focusing their efforts to map, identify, and clone a number of genes responsible for inherited eye disorders. Significant progress has already being achieved for a number of eye diseases, including retinitis pigmentosa (RP), for which >30 different loci are now thought to be participating in the etiology of the condition (Dryja and Li 1995). However, very little work has been done on the molecular genetic study of glaucoma, until recently. Juvenile-onset POAG has been mapped to the 1q21-q31 region

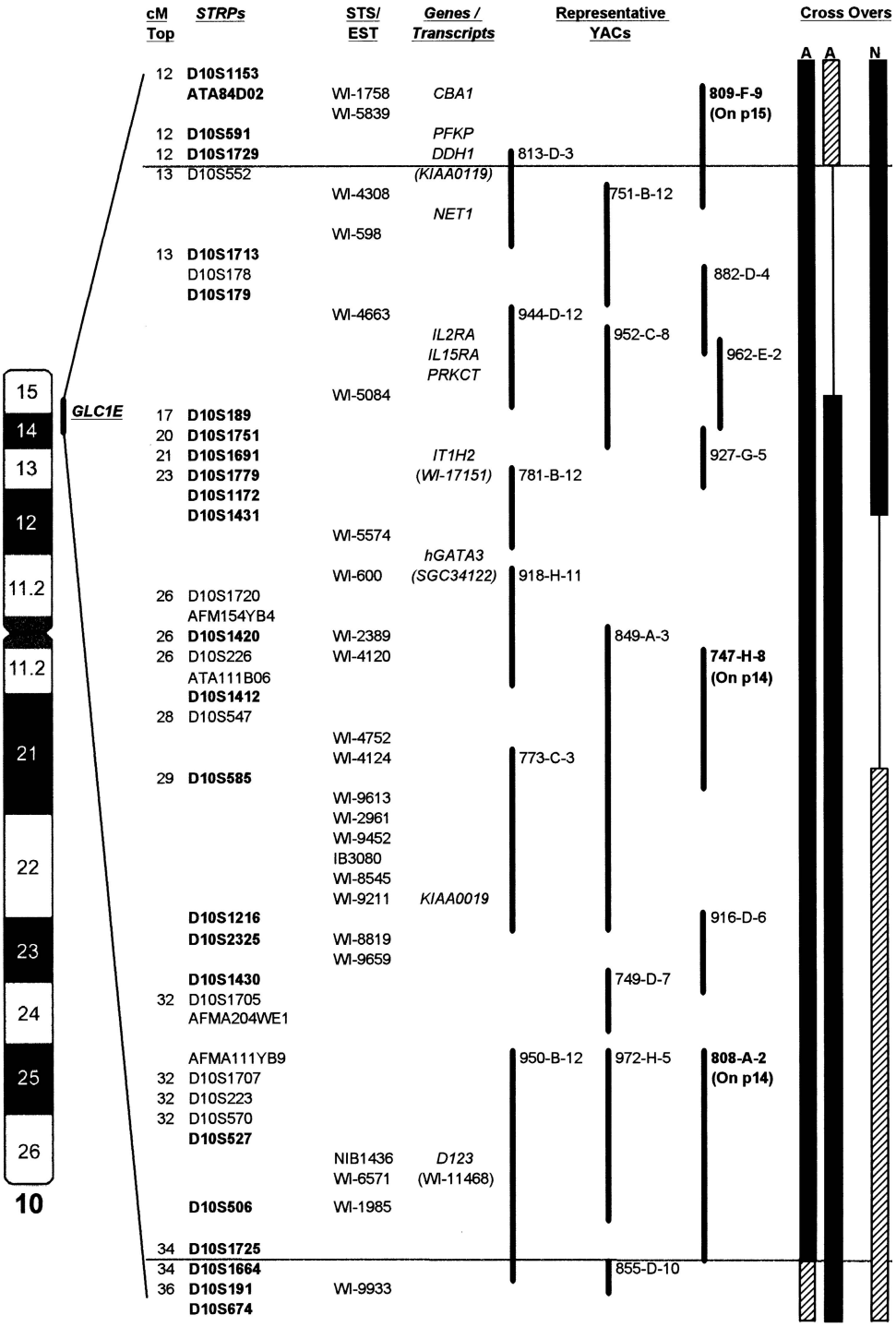

Figure 5 Chromosomal location, linkage, and partial physical map of the GLC1E locus. On the far left part of this figure, the approximate location of this locus on p15-p14 is indicated. This is followed by a column showing the accumulative recombination fractions from the top of this chromosome. Next, the composite order of the STRP markers is based on the published linkage and radiation hybrids maps as well as the YAC contigs and physical mapping data. The STRP markers genotyped in the POAG-90 family are shown in boldface. The next column shows approximate locations of the STS and EST markers, based on the data obtained from the Whitehead Institute for Genome Research. The locations of genes and other transcripts in the next column are based on data obtained from both the Whitehead and Stanford radiation mapping data. A selective number of YACs covering the GLC1E candidate region is also shown. Note that YAC 809-F-9 maps to p15 and YACs 747-H-8 and 808-A-2 to p14. On the far right of this figure, the observed crossovers in two affected individuals (A), III:14 and IV:11, are shown with two solid-black bars. The last bar represents the crossover in the normal individual (N), III:23. The nonrecombinant portions of these three chromosomes are shown with hatched bars. For individual IV:11 (second black bar), the thin black bar indicates that the exact site of recombination between D10S552 and D10S189 is unknown. Similarly, the thin portion of the last bar, for normal individual III:23, indicates that the exact site of recombination between the two markers D10S1172 and D10S585 is unknown.

(Sheffield et al. 1994; reviewed by Raymond [1997] and Sarfarazi [1997]), and, recently, it has been shown to be the result of mutations in the TIGR gene (Adam et al. 1997; Stoilova et al. 1997; Stone et al. 1997). Similarly, three other loci for COAG have been mapped to the 2cen-q13 (GLC1B; Stoilova et al. 1996), 3q21-q24 (GLC1C; Wirtz et al. 1997), and 8q23 (GLC1D; Trifan et al., in press) regions. However, there is now evidence that these four loci are heterogeneous, and additional loci are expected to be involved in the etiology of this group of eye disorders. In our continuing efforts to map and identify gene loci responsible for different glaucoma phenotypes, in addition to the GLC1B locus on 2cen-q13 and GLC1D on 8q23, we have mapped two other loci (GLC3A on 2p21, Sarfarazi et al. 1995; GLC3B on lp36, Akarsu et al. 1996) for primary congenital glaucoma (Buphthalmos) and, more recently, have shown that GLC3A (MIM 231300) on 2p21 is due to mutations in the cytochrome P4501B1 (CYP1B1; MIM 601771) gene (Stoilov et al. 1997, 1998 [in this issue]). The congenital form of glaucoma, like POAG, is also heterogeneous, and there is evidence that other loci are also involved in the etiology of this infantile condition. Therefore, what has already been observed for RP may well be anticipated for glaucoma. The mapping of the first three loci for late-onset POAG has been an opening ceremony to a large number of loci that are predicted to be involved in this group of eye disorders.

In this study, we used a very well defined and characterized single large COAG family and searched the genome to identify a locus for this condition. Our initial strategy of searching for linkage to all the known glaucoma loci, as well as to other potential candidate gene loci, was not successful, and, therefore, we embarked on a random genomewide search to identify the putative locus in this family. After genotyping >95 microsatellite markers, we detected linkage with a DNA marker from the 10p15-p14 region. Subsequent analysis with 23 markers from the same region confirmed the initial linkage in this family. Our analysis indicated that this locus (designated "GLC1E") is tightly linked to D10S1216, with $Z_{max} = 10.00$ for all the meioses or a corresponding value of $Z_{max} = 3.43$ at $\theta = .00$ when only the affected meioses were used. The GLC1E locus is found to be flanked by D10S1729 telomerically ($Z_{max} = 5.55$; $\theta = .04$) and by D10S1664 ($Z_{max} = 6.04$; $\theta = .03$) centromerically, within an estimated genetic distance of ~21 cM. Of a total of 42 affected and normal members of this family, 39 subjects have inherited the appropriate haplotype compatible with their previously classified clinical phenotype. The other three subjects are still too young to show any sign of the condition.

The clinical manifestation in most of the affected subjects of this family represents a classical NTG with cupping of the ONH and visual field loss, in the absence of increased IOP. Inspection of 15 affected members of this family revealed a lower mean age at detection of 43 years (range 23–65 years) in 12 subjects classified by definition as NTG, as compared with the 3 others defined as COAG, with an average age of 48 years (range 38–62 years). The average IOP for family members with NTG was 17.58 mmHg (range 14–21 mmHg) untreated and 14 mmHg (range 6–17 mmHg) treated, whereas in the COAG members it was 23 mmHg (range 21–25 mmHg) untreated and l3 mmHg (range 10–18 mmHg) treated, with a diurnal variation of 5–6 mmHg. The cup/disc ratios averaged 0.78 (range 0.60–0.95) in patients with NTG and 0.80 (range 0.50–0.90) in the COAG patients. Although some ocular and systemic findings such as myopia, systemic hypertension, vascular disease, and migraine are reported to be unusually common in the NTG patients (Werner 1996), we could not prove any association between these and the NTG phenotype in the family investigated here. There is clearly much clinical overlap between COAG and NTG, if indeed it is basically justifiable to separate POAG into these two components. Genetic determination of families such as the one described in this report, together with DNA genotyping, will almost certainly lead to more coherence in the subclassification of POAG.

A number of genes that are known to be located in this region of chromosome 10 could positionally be considered as candidates involved in the etiology of this particular POAG family. A partial list of these genes (fig. 5) includes PFKP (6-phosphofructokinase, type C; MIM 171840), DDH1 (trans-1,2-dihydrobenzene-1,2-diol dehydrogenase), NET1 (guanine nucleotide regulatory protein), IL2RA (interleukin-2 receptor, alpha; MIM 147730), IL15RA (interleukin-15 receptor, alpha; MIM 601070), PRKCT (protein kinase C-theta), ITIH2 (inter-alpha-trypsin inhibitor complex component II; MIM 146648), hGATA3 (trans-acting T-cell specific transcription factor), the mRNA for open reading frame KIAA0019, and the gene for D123 protein. Both PFKP and DDH1 map very close to D10S591, a DNA marker that has recombined in one of the affected subjects (IV:11; fig. 1). After constructing the haplotypes in this branch of the family, we were able to infer the haplotypes of her deceased father (III:15) and to further refine this crossover to between markers D10S1729 and D10S189 (fig. 5). Therefore, the last two genes are probably excluded in this family. Furthermore, physical mapping data suggest that NET1, IL2RA, IL15RA, and PRKCT lie within this uninformative region. Therefore, it is likely that all or at least some of these genes are also excluded, depending on the exact position of recombination in this region. ITIH2 is a part of the inter-alpha-trypsin inhibitor (ITI) complex (Gebhard et al. 1988). ITI is a human serum protease inhibitor of molecular mass 240 kD, which may release physiological deriva-

tives, and has been shown to interact with hyaluronic acid, resulting in pericellular matrix stabilization (Kobayashi et al. 1996). This is a potential candidate gene for the phenotype observed in this family. Mapped between D10S189 and D10S1664 are hGATA3, the mRNA for open reading frame KIAA0019, and the D123 protein. Their potential involvement in the etiology of this phenotype remains to be investigated.

A homologue of the above-mentioned genes within the GLC1E candidate region has already been mapped to the mouse chromosome 2; the homologue of this phenotype is expected to be on the same mouse chromosome. There are currently no known genes in the mouse that can be considered as the homologue of the GLC1E. Further work is currently in progress to search for mutation in a number of these genes and eventually to ascertain the identity of the defective gene for the 10p15-p14–1inked POAG family.

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