

1,4,5-trisphosphate, and inositol-1,3,4,5-tetrakisphosphate were prepared in 10 mM Hepes (pH 7.4). Inositolphosphates and phospholipids were added to bound GST fusion protein on beads together with <sup>32</sup>P-labeled phosphoinositides. Bound lipids were resolved by TLC.

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20. Abbreviations for the amino acid residues are as

follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

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## Identification of a Gene That Causes Primary Open Angle Glaucoma

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Glaucoma is a major cause of blindness and is characterized by progressive degeneration of the optic nerve and is usually associated with elevated intraocular pressure. Analyses of sequence tagged site (STS) content and haplotype sharing between families affected with chromosome 1q-linked open angle glaucoma (*GLC1A*) were used to prioritize candidate genes for mutation screening. A gene encoding a trabecular meshwork protein (*TIGR*) mapped to the narrowest disease interval by STS content and radiation hybrid mapping. Thirteen glaucoma patients were found to have one of three mutations in this gene (3.9 percent of the population studied). One of these mutations was also found in a control individual (0.2 percent). Identification of these mutations will aid in early diagnosis, which is essential for optimal application of existing therapies.

In the United States, glaucoma is the second leading cause of legal blindness overall and the leading cause of blindness in African-American individuals (1, 2). Primary open angle glaucoma (POAG) is the most common form of glaucoma, affecting 1 to 2% of the population over age 40 (3). Nearly 12,000 people in the United States are blinded annually by this disorder (2-4). The molecular basis of POAG is unknown, although it is likely to be a genetically

heterogeneous disorder that results from the interaction of multiple genes and environmental influences.

One method of identifying genes involved in multifactorial disorders is to study Mendelian diseases with a similar phenotype. Juvenile open angle glaucoma (JOAG) is a term used to refer to a subset of POAG that has an earlier age of onset and a highly penetrant autosomal dominant mode of inheritance (5). On clinical examination, patients with juvenile-onset open angle glaucoma are identical to patients

with later onset disease in that both groups exhibit elevated intraocular pressure and optic nerve cupping in the presence of a biomicroscopically normal trabecular meshwork. A genetic locus (*GLC1A*) associated with JOAG was identified on chromosome 1q21-q31 by genetic linkage analysis (6). A number of other groups subsequently identified additional families in which JOAG mapped to this locus (7-9). Observed recombinations between the glaucoma phenotype and highly polymorphic genetic markers in two large JOAG kindreds allowed the interval containing the *GLC1A* gene to be narrowed to a 3-centimorgan region of chromosome 1q between markers D1S3665 and D1S3664 (10). Further evaluation of marker haplotypes revealed that each of three pairs of glaucoma families shared alleles of the same eight contiguous markers, suggesting that *GLC1A* lies within a narrower interval defined by D1S1619 and D1S3664 (11) (Fig. 1).

Several genes mapping to the *GLC1A* region of chromosome 1 were considered as candidates for the disease-causing gene. Three genes [*LAMC1* (12), *NPR1* (13), and *CNR2* (14)] were excluded from the candidate region by genetic linkage analysis with intragenic polymorphic markers (10). Five additional candidate genes were determined to lie within the observed recombinant interval by yeast artificial chromosome (YAC) sequence tagged site (STS) content mapping: selectin E (*SELE*) (15) (GenBank accession number M24736); selectin L (*SELL*) (16) (GenBank accession number

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**Table 1.** Prevalence of *GLC1A* gene mutations.

Mutation	Familial glaucoma* (n = 227)	Unselected glaucoma† (n = 103)	General population‡ (n = 380)	Normal volunteers§ (n = 91)
Gly357Val	2 (0.9%)	0 (0%)	0 (0%)	0 (0%)
Gln361STOP	6 (2.6%)	3 (2.9%)	1 (0.3%)	0 (0%)
Tyr430His	2 (0.9%)	0 (0%)	0 (0%)	0 (0%)
Totals	10 (4.4%)	3 (2.9%)	1 (0.3%)	0 (0%)

\*Unrelated probands having at least one living first-degree relative with a documented history of glaucoma.

†Unrelated, consecutive patients with open angle glaucoma seen in the University of Iowa glaucoma clinic.

‡Unrelated patients with retinal disease (n = 333) and unrelated spouses from previous linkage studies (n = 47). These patients were collected without regard to personal or family history of glaucoma and were used as an approximation of the general population.

§Unrelated volunteers 40 years of age or older who do not have a personal or family history of glaucoma and whose intraocular pressures were less than 20 mm Hg.

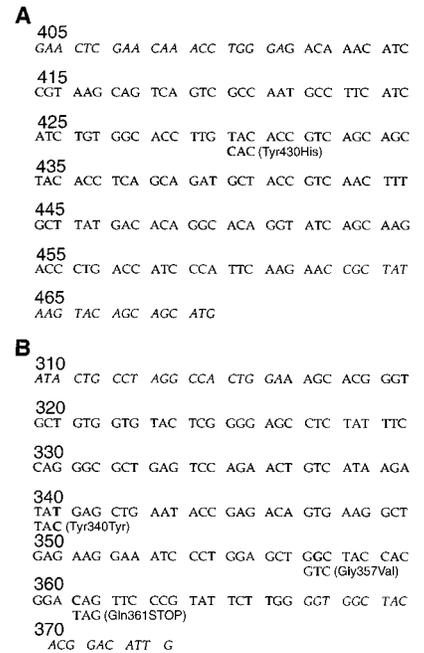
M25280); *TXGP1* (17) (GenBank accession number MD90224); *APTILG1* (18); and *TIGR* (trabecular meshwork-induced glucocorticoid response protein) (19–21) (GenBank accession numbers R95491, R95447, R95443, R47209, and U85257). However, two of these genes (*SELE* and *SELL*) were found to lie outside of the shared haplotype interval with this approach (Fig. 1). The remaining genes (*APTILG1*, *TXGP1*, and *TIGR*) were found to map within the narrowest JOAG interval by both YAC STS content and radiation hybrid mapping (22) (Fig. 1). Two of these genes (*APTILG1* and *TIGR*) were screened for mutations in families with JOAG. Primers were selected from available sequences (18, 20, 21) (GenBank accession numbers R95491, R95447, R95443, R47209, and U85257), and overlapping polymerase chain reaction (PCR) amplification products were evaluated by single-strand conformation polymorphism (SSCP) analysis (23, 24) and direct DNA sequencing (25). Although the complete cDNA sequence of the *APTILG1* (18) and *TIGR* (21) genes are known, the presence of intervening sequences permitted only 85 to 90% of their coding sequences to be screened in genomic DNA. Eight unrelated JOAG patients were screened with the *APTILG1* assay, but no sequence variants were identified.

The *TIGR* gene assay was initially used to screen affected members of four different chromosome 1q-linked glaucoma families and affected members of four smaller families implicated by haplotypic data. Amino acid-altering mutations were detected in five of the eight families. A tyrosine-to-histidine mutation in codon 430

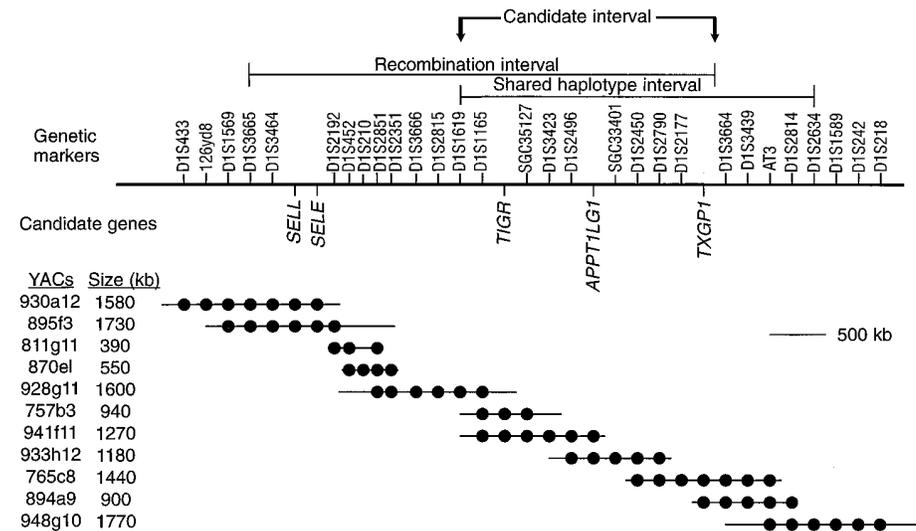
(Tyr430His) (Figs. 2A and 3) was detected in all 22 affected members of the original family (6) linked to chromosome 1q. A glycine-to-valine mutation in codon 357 (Fig. 2B) was detected in two families including one previously unreported adult-onset open angle glaucoma family with 15 affected members (Fig. 3). A nonsense mutation (glutamine to stop) at codon 361 (Figs. 2B and 4) was detected in two families. The latter mutation would be expected to result in a 136-amino acid truncation of the gene product.

The prevalence of mutations in the two PCR amplification products of the *TIGR* gene that harbor these three changes was then estimated by screening four different populations: glaucoma patients with a family history of the disease; unselected POAG probands seen in a single clinic; the general population (approximated by patients with heritable retinal disease and spouses from families who participated in prior linkage studies); and unrelated volunteers over the age of 40 with normal intraocular pressures and no personal or family history of glaucoma (Table 1). PCR products determined to contain a sequence variation by SSCP were sequenced (25) and compared with sequence generated from an unaffected individual as well as the normal chromosome in each affected individual. These experiments revealed eight additional individuals harboring the Gln361STOP mutation and one additional individual harboring the Tyr430His mutation. Also, a tyrosine-to-tyrosine wobble polymorphism in codon 340 was detected in 2.5 to 6.5% of the four patient groups. No amino acid-altering mutations were detected in individuals known

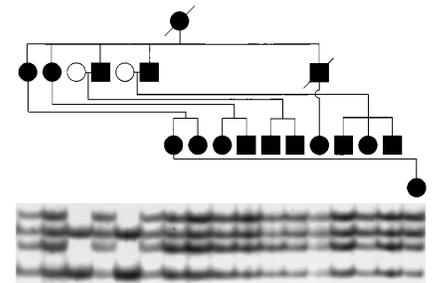
to be free of glaucoma (normal volunteers), but a Gln361STOP mutation was detected in a single person from the general population group. This Gln361STOP mutation was also found in 3 of 103 consecutive unrelated open angle glaucoma patients seen in a glaucoma clinic, which suggests that the *GLC1A* gene is involved in a



**Fig. 2. (A and B)** Two PCR products of the *GLC1A* gene found to harbor sequence changes in patients with glaucoma. The codon numbers correspond to those in (21). The oligonucleotide primer sequences used to amplify each segment are shown in italics. The altered nucleotides are shown in bold below the normal sequence.



**Fig. 1.** Yeast artificial chromosomes composing the minimum tiling path contig are shown. Solid circles indicate the position of markers (sequence tagged sites) that were shown to be contained within each YAC. Disease intervals based on recombination within families and shared haplotypes between families are indicated with brackets.



**Fig. 3.** Segregation of the Gly357Val mutation in a four-generation family affected with open angle glaucoma. Black symbols in the pedigree drawing indicate individuals with documented evidence of open angle glaucoma. White symbols indicate spouses who are clinically unaffected. A photograph of a silver-stained SSCP gel (23, 24) is shown below the pedigree drawing and is aligned so that each gel lane lies directly below the pedigree symbol of the family member whose DNA was analyzed in that lane. For simplicity, clinically unaffected family members were not included in the figure.

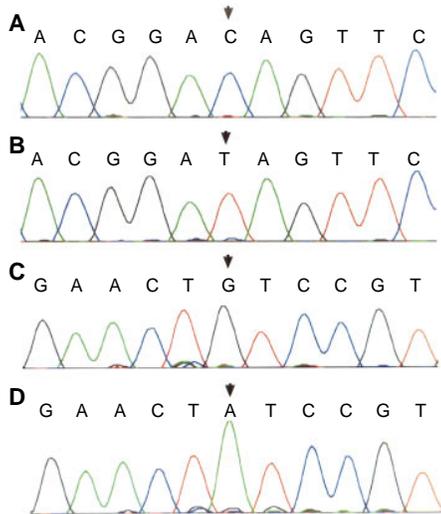
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significant fraction of all glaucoma—not just the subset with a strong family history. Overall, missense or nonsense mutations were found in 13 of 330 unrelated glaucoma patients (3.9%) and 1 of 471 controls (0.2%). A  $\chi^2$  test revealed this difference to be significant (probability  $P < 0.001$ ).

In summary, we have used genetic linkage analysis and examination of shared haplotypes to narrow the glaucoma disease interval known to be on chromosome 1q. We examined a gene (*TIGR*) that lies within the *GLC1A* interval and is known to be expressed in the ciliary body (20) and trabecular meshwork (19, 21), and we found 13 unrelated patients with glaucoma [including the proband of the family (6) whose glaucoma phenotype was originally linked to chromosome 1q] who each harbored one of three different amino acid–altering mutations. Collectively, this is compelling evidence that mutations in *TIGR* are responsible for the glaucoma previously linked to chromosome 1q (*GLC1A*). In addition, the discovery of a mutation in 15 affected members of an adult-onset family, as well as the identification of mutations in 2.9% of a consecutively ascertained group of unselected open angle glaucoma patients, suggests that this gene plays a role in a portion of all open angle glaucoma. It is possible that more than 3% of open angle glaucoma will eventually be shown to be associated with mutations in this gene for two reasons: (i) only a portion of the gene was evaluated

in this study, and (ii) the screening methods used are not 100% sensitive.

The identification of any disease gene has the potential to increase our understanding of the pathophysiology of the disease, which in turn could lead to more effective treatments. The *TIGR* gene product has been proposed to cause increased intraocular pressure by obstruction of aqueous outflow (26). Its expression in trabecular meshwork and ciliary body (structures of the eye involved in the regulation of intraocular pressure) is consistent with this hypothesis. It will now be possible to investigate whether the mutations described here act through this mechanism. Identification of a disease gene also provides the possibility of developing accurate, inexpensive presymptomatic testing for the predisposition to the disease. The value of such testing is limited when the disease is very rare or when there is no currently effective treatment. However, the prevalence of the sequence changes observed in this study, coupled with the prevalence of glaucoma in the general population (2–4), suggests that mutations in *GLC1A* cause glaucoma in nearly 100,000 individuals in the United States. This would make *GLC1A* the most common molecularly recognizable form of blindness. For comparison, only 2000 people in the United States would be expected to harbor mutations in the rhodopsin gene, which is the most common form of molecularly recognizable retinitis pigmentosa. Open angle glaucoma can be successfully treated with existing drugs or surgical approaches in the majority of cases (27). The main difficulty is in diagnosing this silent disease before irreversible optic nerve damage has occurred. The discovery of specific glaucoma-causing mutations will make it possible to identify individuals at high risk for this form of glaucoma before significant visual loss has occurred and to direct them toward sight-saving therapy.



**Fig. 4.** Representative chromatograms generated by fluorescent dye–primer sequencing (25) of cloned PCR amplification products from an affected individual reveal a C-to-T transition, which would be expected to result in a premature stop at codon 361 (Gln361STOP). Sequences in the forward and reverse directions for both the mutant and normal gene are shown: (A) normal, forward; (B) mutant, forward; (C) normal, reverse; and (D) mutant, reverse.

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22. YAC-STS content analysis was performed by PCR amplification of each STS from DNA prepared from individual YAC clones. PCR products were electrophoresed on 2% agarose gels and stained with ethidium bromide. The presence or absence of amplification was scored and this data used to deduce the minimum tiling path of the chromosome 1q glaucoma interval. Radiation hybrid mapping was performed with the Genebridge 4 radiation hybrid panel available from Research Genetics. DNA from each radiation hybrid clone was amplified in duplicate with STS primers. PCR products were electrophoresed on 2% agarose gels and visualized with ethidium bromide. The gels were scored for the presence of amplified product. Radiation hybrid maps based on this data were constructed with the computer programs RH2PT, RHMAXLIK, and RHMINBRK [K. Lange, M. Boehnke, D. R. Cox, K. L. Lunetta, *Genome Res.* **5**, 136 (1995)].
23. A 12.5-ng sample of each patient's DNA was used for the template in an 8.35- $\mu$ l PCR mixture containing 1.25  $\mu$ l of 10X buffer (100 mM tris-HCL, pH 8.3, 500 mM KCl, 15 mM MgCl<sub>2</sub>); the deoxynucleotides dCTP, dATP, dGTP, and dTTP (300  $\mu$ M for each); 1 pmol of each primer; and 0.25 units of *Taq* polymerase (Boehringer Mannheim). Samples were denatured for 5 min at 94°C and incubated for 35 cycles under the following conditions: 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s in a DNA thermocycler (Omnigene). After amplification, 5  $\mu$ l of stop solution (95% formamide, 10 mM NaOH, 0.05% bromophenol blue, 0.05% xylene cyanol) was added to each sample. Amplification products were denatured for 3 min at 94°C and electrophoresed on 6% polyacrylamide–5% glycerol gels at 25 W for about 3 hours. After electrophoresis, gels were stained with silver nitrate (24).
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