CLINICAL FEATURES ASSOCIATED WITH MUTATIONS IN THE CHROMOSOME 1 OPEN-ANGLE GLAUCOMA GENE (*GLC1A*)

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ABSTRACT

Background A substantial proportion of cases of glaucoma have a genetic basis. Mutations causing glaucoma have been identified in the chromosome 1 open-angle glaucoma gene (*GLC1A*), which encodes a 57-kd protein known as myocilin. The normal role of this protein and the mechanism by which mutations cause glaucoma are not known.

Methods We screened 716 patients with primary open-angle glaucoma and 596 control subjects for sequence changes in the *GLC1A* gene.

Results We identified 16 sequence variations that met the criteria for a probable disease-causing mutation because they altered the predicted amino acid sequence and they were found in one or more patients with glaucoma and in less than 1 percent of the control subjects. These 16 mutations were found in 33 patients (4.6 percent). Six of the mutations were found in more than 1 subject (total, 99). Clinical features associated with these six mutations included an age at diagnosis ranging from 8 to 77 years and maximal recorded intraocular pressures ranging from 12 to 77 mm Hg.

Conclusions A variety of mutations in the *GLC1A* gene are associated with glaucoma. The spectrum of disease can range from juvenile glaucoma to typical late-onset primary open-angle glaucoma. (N Engl J Med 1998;338:1022-7.)

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GLAUCOMA is a disorder of the optic nerves that is characterized by cupping of the optic-nerve head and loss of peripheral vision. Occasionally, there is also loss of central vision. Intraocular pressure is elevated in the majority of cases and is thought to contribute to the optic-nerve damage. The disease is insidious, and affected patients frequently have no symptoms. In over 90 percent of patients with glaucoma, the trabecular meshwork appears to be completely normal on clinical examination, and as a result, such patients are said to have open-angle glaucoma. The age at onset of open-angle glaucoma ranges from less than 10 years to more than 70 years, with the majority of cases occurring after the age of 40. When detected early, most cases can be successfully treated with medications, laser treatment, or surgery. Glaucoma is the second leading cause of blindness in developed countries.[1] Its prevalence increases with age and is higher among blacks than among whites.[1] Primary open-angle glaucoma affects 1 to 2 percent of the population over the age of 40.[1]

A substantial fraction of the cases of glaucoma have a genetic basis,[2-11] which allows genetic methods to be used to investigate the pathophysiologic mechanisms of the disease at the molecular level. The chromosomal locations of genes causing three genetically distinct types of primary open-angle glaucoma have been identified.[12-17] Recently, Stone et al.[18] identified three mutations in a gene that lies within the interval on chromosome 1 originally associated with juvenile open-angle glaucoma (*GLC1A*).[12] The *GLC1A* gene encodes a 57-kd protein, myocilin, that is expressed in a number of human tissues.[19-21] The normal role of myocilin and the mechanism by which mutations in this gene cause glaucoma are not known. In this paper, we describe 13 additional *GLC1A* mutations in patients with open-angle glaucoma and summarize the clinical features associated with 6 of these mutations.

METHODS

The study was approved by the human-subjects review committee at the University of Iowa, and written informed consent was obtained from all study participants. Primary open-angle glaucoma was defined as the presence of an intraocular pressure of more than 21 mm Hg as well as evidence of glaucomatous damage to the optic-nerve head. Visible damage to the optic-nerve head alone was accepted as evidence if there was documented enlargement of the cup of the optic-nerve head. Otherwise, both an enlarged cup with a thin neural rim and characteristic visual-field loss related to the optic nerves were required. Patients were excluded if they had a history of eye surgery before the diagnosis of glaucoma or evidence of secondary glaucoma, such as exfoliation or pigment dispersion. We recruited as control subjects people who were over 40 years of age, had intraocular pressures below 20 mm Hg, and had no family or personal history of glaucoma.

We used single-strand conformation polymorphism (SSCP) analysis to screen unrelated patients with primary open-angle glaucoma and control subjects for mutations in the coding sequence of the *GLC1A* gene. This technique is capable of identi-

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fying over 90 percent of single-base changes within a gene as long as the gene is analyzed in fragments of 200 bp or less.[22] The sequences of the oligonucleotide primers used for the *GLC1A* assay are given in the Appendix. Mutations were confirmed by automated DNA sequencing as previously described.[18] Relatives of probands who were found to have sequence changes in GLC1A were also evaluated for mutations. Efforts were made to examine or review the medical records of all family members found to have mutations.

The age at diagnosis of primary open-angle glaucoma and the highest recorded intraocular pressures associated with six different mutations were evaluated with Kruskal–Wallis nonparametric analysis of variance.[23] All P values were two-tailed. In the four families with the largest numbers of affected members, we used linkage analysis to determine whether the *GLC1A* mutation cosegregated with the disease phenotype. Briefly, the segregation of alleles of genetic markers at the *GLC1A* locus was determined for the clinically affected members of each family. Then, the probability of this segregation was determined for two different conditions: physical linkage of the marker and disease gene and physical independence of the marker and disease gene. The lod score is the logarithm of the ratio of the probability that the condition is linked to the probability that the condition is not linked. A lod score of 3 signifies that the odds in support of linkage are 1000 to 1 and is the accepted threshold for significance. Pairwise linkage analysis was performed with the MLINK and LODSCORE programs as implemented in FASTLINK (version 2.3),[24,25] part of the LINKAGE program package.[26] A person's disease status was considered to be unknown unless the clinical features of primary open-angle glaucoma (as defined above) were present. The frequency of the mutant allele was assumed to be 1 percent in all cases.

RESULTS

Characteristics of the Study Subjects

A total of 1446 subjects were studied: 716 unrelated patients (348 males and 368 females) with primary open-angle glaucoma (the probands), 96 subjects with primary open-angle glaucoma who were relatives of the probands, 38 clinically unaffected siblings of a proband or affected family member, 505 subjects from the general population, and 91 normal subjects known to be free of glaucoma. The average age of the 716 probands was 67.1 years. Five hundred sixty-three of the probands were from Iowa, 97 were from Australia, and the other 56 were from elsewhere in the United States. Among the probands from Iowa, 132 (23 percent) were identified on the basis of a family history of glaucoma, and 431 (77 percent) were consecutively identified at the University of Iowa glaucoma clinic. The subjects from the general population were used to determine the approximate population frequency of the sequence changes observed in the study, and no information was available about the presence or absence of glaucoma in these subjects. Of these subjects, 184 were from Iowa, 210 were from elsewhere in the United States, 79 were from Europe, 19 were from Canada, and 13 were from Australia. All 91 normal subjects were from Iowa. All groups had similar ethnic distributions, and similar proportions (more than 85 percent) were white. A portion of the *GLC1A* gene had previously been evaluated for mutations in 330 of the probands, 380 of the subjects from the general population, and all 91 normal subjects.[18] In this study, the entire coding region was evaluated.

Identification of the Mutations

Screening with SSCP analysis followed by sequencing of DNA from 1312 unrelated subjects (the probands, the subjects from the general population, and the normal controls) identified a total of 35 sequence changes in *GLC1A*. Sequencing of the entire coding region of *GLC1A* amplified from the probands of three families with glaucoma linked to chromosome 1q but without abnormal SSCPs revealed three additional sequence changes. Sixteen of these 38 sequence variations (Table 1) met the following criteria for a probable disease-associated mutation: they were present in 1 or more patients with glaucoma and in less than 1 percent of the general population, they altered the predicted amino acid sequence, and they were absent in the 91 normal subjects. These 16 mutations were found in 33 of the 716 probands with glaucoma (4.6 percent). Ten of the 38 sequence changes altered the predicted amino acid sequence of *GLC1A* and 1 altered the 5′ flanking region, but they were judged unlikely to be disease-causing mutations (Table 2) for one of the following reasons: 3 were present in more than 1 percent of the general population, 7 were found in the general population at a frequency greater than the frequency in the population with glaucoma, and 1 was found in the same allele as a mutation more likely to cause disease. Eleven of the 38 sequence changes did not alter the predicted amino acid sequence of *GLC1A* and therefore were also judged unlikely to be disease-causing mutations (Table 3).

Effects of the Mutations

The effect of each of the 26 changes that alter the amino acid sequence on the polarity, charge, and size of the predicted gene product was evaluated. All but four of the changes that altered polarity, charge, or size were judged to be probable disease-causing mutations (Table 1). Collectively, these mutations were found in 29 of 716 probands (4.1 percent) and 5 of 596 control subjects (0.8 percent) ($P<0.001$). When we repeated this analysis using only the 563 probands, 91 normal subjects, and 184 subjects from the general population who were from Iowa (who were therefore more likely to be of similar ethnic origin), mutations causing changes in the polarity, charge, or size of the predicted gene product were found in 24 of the probands (4.3 percent) and 2 of the control subjects (0.7 percent) ($P=0.005$).

Frequency of the Mutations

Six mutations were found in more than one patient with glaucoma. These six mutations were found in 23 of the 716 probands. Analysis of the rel-

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TABLE 1. MUTATIONS IDENTIFIED IN THE *GLC1A* GENE THAT WERE JUDGED PROBABLE DISEASE-CAUSING MUTATIONS.

MUTATION	MAXIMAL LOD SCORE (θ)*	EFFECT†	PROBANDS AND AFFECTED RELATIVES (N=812)	PROBANDS (N=716)	NORMAL SUBJECTS (N=91)	SUBJECTS FROM THE GENERAL POPULATION (N=505)
			number			
Gln19His	—	Change in charge	1	1	0	0
Arg82Cys	—	Change in charge	1	1	0	0
Trp286Arg	—	Change in charge	1	1	0	0
Thr293Lys	—	Change in charge	1	1	0	0
Pro361Ser	—	Change in polarity	1	1	0	0
Gly364Val‡	3.5 (0.05)	None	20	2	0	0
Gln368STOP‡	—	Premature termination	25	15	0	1
Thr377Met	1.3 (0.2)	Change in polarity	15	2	0	0
Asp380Gly	—	Change in charge	1	1	0	0
396INS397	—	Multiple	6	1	0	0
Arg422His	—	None	1	1	0	0
Tyr437His‡	13.8	Change in charge	34	2	0	0
Ala445Val	—	None	1	1	0	0
Arg470Cys	—	Change in charge	1	1	0	0
Ile477Asn	11.6	Change in polarity	19	1	0	0
Lys500Arg	—	None	1	1	0	0

*Unless otherwise indicated in parentheses, θ equaled 0.

†"None" signifies an amino acid change that did not alter the charge, size, or polarity of the predicted gene product, and "Multiple" signifies that multiple residues were affected by the mutation (not including premature termination).

‡The codon numbering used for this mutation differs by seven codons from that reported by Stone et al.[18] because there was an error in the complementary DNA sequence originally submitted to GenBank by Nguyen, Polansky, and Huang.

atives of these probands identified an additional 96 patients with the clinical diagnosis of glaucoma and a mutation in the *GLC1A* gene. Complete clinical data were available for 99 of these 119 probands and family members (Table 4). The age at diagnosis ranged from 8 to 77 years, and the highest intraocular pressure recorded in each eye ranged from 12 to 77 mm Hg. A pressure of 12 mm Hg was recorded in the apparently unaffected eye of one of two asymmetrically affected patients. With a Kruskal–Wallis nonparametric analysis of variance,[23] the null hypothesis that the phenotypic differences associated with the different mutations were due to chance was rejected ($P<0.001$) for both the age at onset of glaucoma and the highest recorded intraocular pressure. Thirty-eight unaffected siblings of clinically affected patients with these six mutations were also screened for mutations, and 12 had the mutation that had been identified in their family. Thus, 119 of 131 patients and subjects with a mutation (91 percent) had clinical signs of glaucoma.

The four families with the largest numbers of affected members had maximal lod scores ranging from 1.3 to 13.8 (Table 1). Most kindreds with the Gln368STOP mutation had only a single affected member and were therefore not suitable for conventional linkage analysis. For this mutation, we compared its frequency in probands (15 of 716) and control subjects (1 of 596) using Fisher's exact test; the prevalence of the mutation was significantly higher in patients than in controls ($P=0.001$).

A total of 33 nuclear families had one of the probable disease-causing mutations (Table 1). Twelve had more than one family member with the clinical diagnosis of primary open-angle glaucoma. In four families, one of the clinically affected patients did not have the *GLC1A* mutation that was present in the other family members. In a fifth family, only a single patient with glaucoma had a *GLC1A* mutation, whereas four other clinically affected relatives did not. In total, 88 of the 96 affected relatives (92 percent) of the probands in these 12 kindreds had the mutation found in the proband.

DISCUSSION

To provide patients who have genetic mutations that may cause disease with useful information about their risk of becoming ill, it is important to establish the disease-causing nature of each sequence variant and the associated penetrance and age at onset of

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TABLE 2. SEQUENCE CHANGES IDENTIFIED IN THE *GLC1A* GENE THAT WERE JUDGED UNLIKELY TO BE DISEASE-CAUSING MUTATIONS.

SEQUENCE CHANGE	FREQUENCY*	EFFECT†	PROBANDS (N=716)	NORMAL SUBJECTS (N=91)	SUBJECTS FROM THE GENERAL POPULATION (N=505)
	%			number	
bp −83 (G→A)‡	18	None	124	21	85
Cys9Ser	0.076	None	0	1	0
Asn73Ser	0.076	None	0	1	0
Arg76Lys§	19	None	NA	17	NA
Ser203Phe	0.076	Change in polarity	0	1	0
Glu352Lys	0.15	Change in charge	1	0	1
Lys398Arg	1.2	None	9	4	3
Arg422Cys	0.076	Change in charge	0	0	1
Ser425Pro	0.076	Change in polarity	0	0	1
Tyr473Cys	0.076	None	0	0	1
Val495Ile¶	0.076	None	1	0	0

*The frequencies of the sequence changes in all 1312 unrelated subjects who were screened for changes in this portion of the gene are given.

†"None" signifies an amino acid change that did not alter the charge, size, or polarity of the predicted gene product.

‡A sequence change was identified 83 bp upstream of the putative start site.

§NA denotes not applicable.

¶This mutation is included because it was found in the same allele as a Thr377Met mutation in two members of a single family.

TABLE 3. POLYMORPHISMS THAT DID NOT ALTER THE PREDICTED AMINO ACID SEQUENCE OF THE *GLC1A* GENE PRODUCT.

SEQUENCE CHANGE	FREQUENCY*	PROBANDS (N=716)	NORMAL SUBJECTS (N=91)	SUBJECTS FROM THE GENERAL POPULATION (N=505)
	%		number	
Pro13Pro	0.53	3	1	3
Gly122Gly	0.53	5	0	2
Leu159Leu	0.38	2	1	2
Thr204Thr	0.076	0	0	1
Lys266Lys	0.076	1	0	0
Thr285Thr	0.69	8	1	0
Thr325Thr	1.1	6	1	8
Val329Val	0.076	1	0	0
Tyr347Tyr	5.4	41	7	23
Glu396Glu	0.15	1	0	1
Val439Val	0.076	0	0	1

*The frequencies of the sequence changes in all 1312 unrelated subjects who were screened for changes in this portion of the gene are given.

disease. However, such information can be difficult to gather for a disease such as glaucoma, which is prevalent, pathophysiologically complex, genetically heterogeneous, and incompletely penetrant. One index of the pathogenicity of a given sequence change is its association with the disease phenotype as compared with its frequency in a control group. For example, in our study, 15 of 716 probands with glaucoma (2 percent) had the Gln368STOP mutation, as compared with only 1 of 596 control subjects ($P=0.004$). Such an analysis can be done only for mutations that are identified in multiple affected patients.

Among families with the Tyr437His or Ile477Asn mutation, the mutation was found in every clinically affected member (Table 4), whereas it was not identified in subjects from the general population. Likelihood analysis performed on the segregation of Tyr437His and Ile477Asn within families revealed the segregation with the disease to be 10^{14} and 10^{12} times greater than would be expected by chance, respectively. The fact that these were the only *GLC1A* sequence changes found in these families provides evidence that they are associated with open-angle glaucoma. In contrast, 10 of the 16 mutations that we identified as probable disease-causing mutations were found in only a single member of a kindred. Support for the pathogenicity of these mutations is limited to the fact that they alter the predicted amino acid sequence of the *GLC1A* gene product and were not found in the normal subjects. One or more of these changes may prove to be rare polymorphisms that do not cause disease. Although the pathogenicity of each rare sequence variant cannot be independently established at this time, the identification of sequence changes that are predicted to result in nonconservative amino acid substitutions in 29 of 716 probands with glaucoma (4.1 percent), as compared with 5 of 596 control subjects (0.8 percent), provides additional evidence that *GLC1A* is a glaucoma-causing gene.

Because primary open-angle glaucoma is a common, genetically heterogeneous disorder, we expected to identify some families in which some affected members did not have the *GLC1A* mutation present in the proband. We identified 8 such persons (phenocopies) among a total of 96 affected relatives of the probands. We also identified some people who have mutations thought to be associated with disease but in whom glaucoma has not yet developed (nonpenetrance). With respect to the Gly364Val, Gln368STOP, Thr377Met, Tyr437His, and Ile477Asn mutations, we believe that enough patients have

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TABLE 4. CLINICAL FEATURES ASSOCIATED WITH SIX *GLC1A* MUTATIONS RELATED TO OPEN-ANGLE GLAUCOMA.

VARIABLE	MUTATION IDENTIFIED IN PROBAND						TOTAL OR RANGE
	GLY364VAL	GLN368STOP	THR377MET	396INS397	TYR437HIS	ILE477ASN	
No. of families	2	15	2	1	2	1	23
No. of affected patients*	16	22	15	6	27	13	99
No. of affected patients without the mutation present in the proband	1	5	1	1	0	0	8
Age at diagnosis of disease (yr)							
Range	22–48	36–77	20–60	19–31	8–41	12–41	8–77
Mean	34	59	37	25	20	21	
Highest intraocular pressure recorded (mm Hg)							
Range	15–65	21–56	20–50	12–60	14–77	20–52	12–77
Mean	36	30	31	40	44	40	

*Values are the numbers of patients with glaucoma and a *GLC1A* mutation for whom data on the age at diagnosis of disease and highest recorded intraocular pressure were available.

been studied to indicate that the penetrance is sufficiently high to state that carriage of these mutations conveys a significant risk of glaucoma. Screening for mutations for which there is strong evidence of pathogenicity and high penetrance may be useful for presymptomatic diagnosis.

We found a wide range in the expression of the various *GLC1A* mutations as well as some predictable correlations between genotype and phenotype. The Tyr437His and Ile477Asn mutations are associated with a form of glaucoma for which the average age at diagnosis is almost four decades earlier than that of the Gln368STOP mutation and in which the intraocular pressure is significantly higher. These observations suggest that at least some of the *GLC1A* mutations act through a dominant negative mechanism rather than simple haploinsufficiency; that is, if all the mutations exert their effect by simply reducing the amount of normal *GLC1A* gene product by half (haploinsufficiency), one would not expect to observe any statistically significant clinical differences between patients with different mutations. In contrast, if the mutant gene product actively participates in the development of disease (a dominant negative mechanism), one would expect to observe significant differences between groups of patients with different mutations, as we did with the six mutations listed in Table 4.

In two unrelated patients with glaucoma — one with a Tyr437His mutation and one with a Thr377Met mutation — there was dramatic asymmetry of the disease: one eye was severely affected, whereas the other eye remained nearly normal. This finding suggests that factors other than the *GLC1A* gene can modify the disease phenotype.

The mechanism by which mutations in the *GLC1A* gene cause increased intraocular pressure and optic-nerve damage is not known. The *GLC1A* gene is expressed in the ciliary body, cultured trabecular-meshwork cells, and the retina.[19-21] It is also expressed in several nonocular tissues, including skeletal muscle, the heart, lungs, and pancreas[19] (and unpublished data). The protein encoded by the *GLC1A* gene has been referred to as myocilin[19] and TIGR (trabecular-meshwork–induced glucocorticoid response)[21] by different groups. The term "myocilin" has recently been adopted by the Human Genome Organization–Genome Database Nomenclature Committee.

In our study, the family with the Ile477Asn mutation is a branch of a family originally investigated by Stokes in 1940.[3] Stokes was able to trace the origin of the disease to an Englishman born in 1799 who was blind by the age of 33. He described five generations of a family in which glaucoma had been diagnosed in 12 males and 9 females at an average age of 25 years. He reported that family members as young as 18 years underwent surgery for glaucoma and that family members as young as 19 were blind. In some family members the disease was diagnosed as late as their early 40s. Some patients had intraocular pressures of more than 60 mm Hg. These observations are in close agreement with the data we collected from the 19 affected patients with the Ile477Asn mutation.

The phenotype of *GLC1A*-associated glaucoma can range from one of a clearly juvenile glaucoma to typical late-onset primary open-angle glaucoma. The prevalence of *GLC1A*-associated glaucoma is high, suggesting that a substantial number of patients may be affected. Because glaucoma is symptomless (until its later stages) but treatable, early detection is im-

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portant. Molecular testing can be used to identify persons with a predisposition to *GLC1A*-associated glaucoma decades before the disease develops. However, for such testing to be of maximal benefit, further understanding of the clinical behavior associated with different *GLC1A* sequence changes will be required.

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APPENDIX

The following table shows the primer sequences used:

Exon	Forward Primer	Reverse Primer
1	5′GGCTGGCTCCCCAGTATATA3′	5′ACAGCTGGCATCTCAGGC3′
1	5′ACGTTGCTCCAGCTTTGG3′	5′GATGACTGACATGGCCTGG3′
1	5′AGTGGCCGATGCCAGTATAC3′	5′CTGGTCCAAGGTCAATTGGT3′
1	5′AGGCCATGTCAGTCATCCAT3′	5′TCTCTGGTTTGGGTTTCCAG3′
1	5′TGACCTTGGACCAGGCTG3′	5′CCTGGCCAGATTCTCATTTT3′
1	5′TGGAGGAAGAGAAGAAGCGA3′	5′CTGCTGAACTCAGAGTCCCC3′
2	5′AACATAGTCAATCCTTGGGCC3′	5′TAAAGACCATGTGGGCACAA3′
3	5′TTATGGATTAAGTGGTGCTTCG3′	5′ATTCTCCACGTGGTCTCCTG3′
3	5′AAGCCCACCTACCCCTACAC3′	5′AATAGAGGCTCCCCGAGTACA3′
3	5′ATACTGCCTAGGCCACTGGA3′	5′CAATGTCCGTGTAGCCACC3′
3	5′TGGCTACCACGGACAGTTC3′	5′CATTGGCGACTGACTGCTTA3′
3	5′GAACTCGAACAAACCTGGGA3′	5′CATGCTGCTGTACTTATAGCGG3′
3	5′AGCAAGACCCTGACCATCC3′	5′AGCATCTCCTTCTGCCATTG3′

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