A Novel Mutation in the GLC1A Gene Causes Juvenile Open-angle Glaucoma in 4 Families From the Italian Region of Puglia

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Background: Primary open-angle glaucoma encompasses a complex of potentially blinding ocular diseases characterized by a normal-appearing angle of the anterior chamber, a characteristic degeneration of the optic nerve with resultant typical visual field defects, and usually, an elevated intraocular pressure. It can be subdivided into 2 groups according to the age at onset: the more prevalent chronic open-angle glaucoma diagnosed after 40 years of age, and the less common juvenile form, which occurs between 3 years of age and early adulthood. A locus for primary open-angle glaucoma (GLC1A) has been mapped to a 3-centimorgan region of the long arm of chromosome 1 (1q23-25). Recently, the myocilin (MYOC) gene, located in this chromosomal interval, has been found mutated in several patients affected by primary open-angle glaucoma.

Objective: To describe the clinical and molecular genetic features of 4 pedigrees affected by autosomal dominant juvenile open-angle glaucoma, all from the Italian region of Puglia.

Methods: Clinical study, gonioscopy, automated perimetry, and DNA analysis were performed on several members of the 4 families.

Results: We identified a new molecular defect (1177GACA→T) in the third exon of the GLC1A gene. This mutation is present in all affected persons and in 2 still phenotypically normal persons.

Conclusion: Our results are important for diagnostic purposes because it is now possible to identify asymptomatic carriers, for whom clinical surveillance for the early detection and treatment of glaucoma may be suggested.


Primary open-angle glaucoma (POAG; catalogued in Mendelian Inheritance in Man 137750) is one of the leading causes of irreversible blindness in developed countries despite efforts in its diagnosis and therapy. Primary open-angle glaucoma probably encompasses a complex of ocular diseases that have in common a normal-appearing angle of the anterior chamber. The disease causes in most patients a higher-than-normal intraocular pressure associated with a progressive degeneration of retinal ganglion cells, leading to atrophy and excavation of the head of the optic nerve and producing the well-known visual field defects. If left unchecked, it may lead to blindness.

The disease is painless, and it progresses slowly; for this reason, it is often diagnosed late, when visual field defects are already severe. Diagnosis at an early stage is desirable to prevent irreversible glaucomatous optic nerve atrophy through medical or surgical therapy.

According to the age at onset, POAG may be subdivided into 2 groups: chronic open-angle glaucoma in patients older than 40 years and juvenile open-angle glaucoma in patients between 3 years of age and early adulthood. There is evidence that at least some forms of juvenile open-angle glaucoma are genetically determined. Hereditary juvenile open-angle glaucoma has a distinct phenotype, and it is inherited as an autosomal dominant trait with high penetrance.

Autosomal dominant juvenile open-angle glaucoma was mapped by linkage analysis to chromosome 1 in the region q21-q31 (official locus name, GLC1A) by Sheffield et al. Subsequent linkage and haplotype analysis of additional families with GLC1A has restricted this region to that bracketed by D1S196 and D1S218 markers. Although Seghatoleslami et al have excluded the association of chronic open-angle glaucoma from the GLC1A region, Morrisette et al described a large multigenerational French Canadian family that shows association with 1q23-25.
PATIENTS AND METHODS

CLINICAL STUDIES

The 4 families described in this article (Figure 1) originate from the Italian region of Puglia. They clearly show autosomal dominant transmission of the disease. Several members of the 4 families underwent clinical examination and contributed blood specimens for genetic evaluation. All subjects gave informed consent before entering the study. Clinical examinations included applanation tonometry, slitlamp biomicroscopy, gonioscopy, ophthalmoscopy, and automated threshold perimetry. Subjects were labeled as POAG-affected if they had a normal open filtration angle (grade 3 or 4) and at least 2 of the following: glaucomatous optic disc damage, glaucomatous visual field loss, and an intraocular pressure of greater than 21 mm Hg.

DNA ANALYSIS

Blood specimens were collected from affected and not affected members of the 4 families. Genomic DNA was extracted using standard procedures. Linkage analysis was carried out using several markers mapped at band 1q21-25 that are closely linked to the GLC1A locus: D1S194, D1S196, D1S445, D1S433, D1S2851, D1S452, D1S210, D1S2815, D1S2790, D1S242, D1S2814, and D1S218. Polymerase chain reaction (PCR) was performed according to standard procedures, and primer sequences were obtained from the Genome Data Bank. For allelic scoring, 2 µL of PCR products were size-fractioned on 6% polyacrylamide gel and autoradiographed. The PCR of the published complementary DNA sequence of the GLC1A gene was performed using the following primers: ATACTGCGTAGGCCACTGGA and CAATGCTCATTAGCCAC. The PCR was performed in 30-µl volumes that included 12 pmol of each primer in solutions of P-dideoxynucleotides, 25 mmol/L; potassium chloride, 50 mmol/L; Tris (pH 9), 10 mmol/L; magnesium chloride, 1.5 mmol/L; and Taq DNA polymerase, 0.5 U. The PCR conditions were as follows: 5 minutes’ denaturation at 94°C, followed by 30 cycles of amplification (30 seconds at 94°C, 30 seconds at 58°C, and 1 minute at 72°C), and a final step of 10 minutes at 72°C. A direct sequence of amplified DNA was performed by cycle sequencing (Thermo Sequenase Kit, Amersham International PLC, Buckinghamshire, England). Both strands were sequenced for each DNA fragment. Specimens were tested for the presence of mutation by dot blot analysis with allele-specific oligonucleotide probes. These probe sequences were as follows: normal, ACCACGGACAGTCC; mutated, ACCACGTCCTCCTG.

LINKAGE ANALYSIS

Linkage analysis was performed using a commercial software version (FASTLINK)14,15 of a linkage analysis computer program (LINKAGE v.5.1, Rice University, Houston, Tex). The 2-point lod scores were calculated using the MLINK and ILINK options of the FASTLINK program. The multipoint analyses were calculated using the LINKMAP option of the FASTLINK program. Linkage significance was evaluated using standard criteria (Zmax>3.0).16

Figure 1. Pedigrees of families A, B, C, and D with primary open-angle glaucoma included in this study. Roman numerals indicate generations. Squares and circles represent males and females, respectively; solid symbols, affected individuals; open symbols, normal individuals; symbols with a slash, deceased members; and asterisk, a person from whom clinical data and the genotype were obtained. In family A, members IV4 and IV5, indicated with a dotted symbol, are asymptomatic carriers.
The patients in this POAG family show great variation in the age at onset, suggesting that other yet-unknown factors beyond the GLC1A gene may influence the phenotype. Recently, new loci for autosomal chronic open-angle glaucoma have been mapped to the 2 centromere q13 region (GLC1B) and to 3q21-24 (GLC1C).

Recently, the gene associated with GLC1A has been identified. This gene encodes for a trabecular meshwork-induced glucocorticoid response protein (TIGR), recently renamed by the official nomenclature as myocilin (MYOC) protein. Mutation analysis allowed the identification of several mutations in a small proportion of patients with POAG. In this article, we present a clinical and genetic analysis of 4 POAG-affected southern Italian families from adjacent villages showing phenotypic variability associated with age-dependent penetrance. The molecular defect found in all these families is a new mutation (1177GACA→T) in the GLC1A gene.

**RESULTS**

<table>
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<tr>
<th>Clinical Data of 4 Italian Families With Primary Open-angle Glaucoma (POAG)*</th>
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<td>Family</td>
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<td>D</td>
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<td>All families</td>
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* IOP indicates intraocular pressure. All patients had a grade 3 or 4 lesion on gonioscopy, and all had abnormal visual fields.

The average highest intraocular pressure (using the highest reading in the eye with the highest pressure) was 37.2 mm Hg. All patients showed an open, normal-appearing angle of the anterior chamber and glaucomatous optic disc damage in 1 or both eyes. Sixteen patients had undergone filtration surgical therapy because the response to medical therapy was not satisfactory; only 4 patients were well compensated with antiglaucomatous topical medication.

**SEQUENCE ANALYSIS**

We first analyzed the region within the third exon where all the mutations published by Stone et al were found. The direct sequence of the amplified PCR product allowed the identification of a new mutation. This molecular defect is caused by the deletion of 4 nucleotides in position 1177 (GACA) and by the insertion of 1 nucleotide (T) (Figure 2). As a result, 2 amino acids—glycine and glutamine (Gly and Gln)—are substituted by valine (Val). All specimens collected were then studied by dot blot analysis with allele-specific oligonucleotide probes for the presence of the 1177GACA→T mutation. This molecular defect is present in all affected and in 2 still unaffacted persons carrying the disease-associated haplotype. All remaining normal members of our families and 100 unrelated people from the same geographical area were tested by dot blot analysis, and the results are negative for this mutation.

**LINKAGE ANALYSIS**

All patients recruited for the clinical study underwent genetic mapping to the autosomal dominant open-angle glaucoma locus by linkage analysis. Family A, with sufficient specimens for a statistical analysis, had lod scores that were positive for markers D1S452, D1S218, and D1S242 (3.38, 3.44, and 3.44, respectively, with a recombination fraction of 0.001). Multipoint analysis confirmed the results obtained by a 2-point analysis. A comparison of shared alleles by the GLC1A haplotypes in affected and not affected members of these families allowed the identification of key recombinants that narrowed the GLC1A critical region between D1S452 and D1S242. In all 4 families, the affected members carry a common haplotype at markers D1S210, D1S2815, and D1S2790 that are associated with the disease, suggesting a common ancestor.
We found an association between the \textit{GLC1A} locus and glaucoma by linkage analysis of 4 autosomal dominant families with POAG originating from the same narrow geographical area. All the affected persons share the same disease-bearing haplotype, clearly indicating a founder effect. The sequence of the \textit{GLC1A} gene allowed the identification of a novel mutation (1177GACA\textrightarrow T) located in the third exon. This mutation produces the loss of 2 amino acids (367Gly and 368Gln), which are replaced by valine, without any further change in the remaining protein. There was a complete concordance between the clinical diagnosis of glaucoma and the genetic detection of the \textit{GLC1A} mutation, but the reverse was not true: 2 unaffected persons were shown to harbor the mutation. The absence of this mutation in 200 normal chromosomes obtained from specimens collected in the same geographical area of our families indicates that this is not a common polymorphism. Further support of this derives from a linkage analysis between the mutation and the disease in our families that gave a positive lod score of 6.62 (at \theta=0.0).

The \textit{GLC1A} gene consists of 3 exons that codify for a 504–amino acid glycoprotein, mainly expressed in the ciliary body, sclera, and trabecular meshwork.\textsuperscript{13} Analysis of the amino acid sequence of MYOC defines at the N terminus a signal peptide, a leucine zipper motif every 7 positions, and 5 arginine residues at every 11 positions that are 25% homologous to the heavy chain of myosin.\textsuperscript{18} This suggests that the MYOC protein is an extracellular matrix component where it forms protein-to-protein interactions. The protein’s C terminus, codified by the third exon, has about 50% homology with rat olfactomedin-related protein, which is an olfactory epithelium-specific extracellular protein.\textsuperscript{18} This domain may be important for protein uptake and metabolism by the trabecular meshwork cells.

Most of the POAG mutations identified so far in the \textit{GLC1A} gene are missense located in the third exon.\textsuperscript{11,19} They may act as a dominant negative mutation leading to MYOC protein accumulation in the extracellular matrix and consequent outflow resistance.\textsuperscript{12} Support of this hypothesis will derive from more functional studies.

The 1177GACA \textrightarrow T mutation is located in a region of the gene homologous to the olfactomedin in a highly conserved amino acid sequence. This confirms that this domain plays a role in the metabolic pathway of this protein.

The clinical phenotype of our cohort of patients appeared to be a severe form of POAG, with the age at diagnosis ranging from 8 to 54 years, and most patients required a filtering operation. The variation in the age at diagnosis and the good response to topical therapy observed in a few patients appear to be true clinical characteristics and are not related to changes in diagnostic criteria. Clinical variation within the same family has been described by other authors, and it reminds us that beyond the correlation between genotype and phenotype, other contributing risk factors may come into play. The MYOC protein is induced by glucocorticoids in an in vitro culture of human trabecular meshwork cells.\textsuperscript{20} The response can be modulated by other factors, however, such as certain basic fibroblast growth factors and nonsteroidal anti-inflammatory drugs. Therefore, different controls of \textit{GLC1A} gene expression may lead to different levels of mutated protein, which would explain the variable age of presentation, ie, the severity of the disease.

\begin{figure}
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\includegraphics[width=\textwidth]{figure2}
\caption{Left, Direct sequencing of the polymerase chain reaction product from affected and normal members of one of the families with primary open-angle glaucoma. Right, Normal and mutated nucleotide sequence from amino acid 365 to 374.}
\end{figure}
Our results are important for diagnostic purposes because it is now possible to identify asymptomatic carriers in whom clinical surveillance for the early detection and therapy of glaucoma may now be suggested.

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REFERENCES


