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.

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: ATACTGCTAGGCCACTGGA and CAATGTCCGTAGCCAC. 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, 30 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, ACCACGAGACCTCC; mutated, ACCACGTGCTCCGT.

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

#### CLINICAL FINDINGS

In the 4 families examined in this study, POAG was transmitted through 4 generations (Figure 1). The members of the first traceable generation, however, are all dead, and their diagnosis is based on family reports that they were blind in the later years of their life. In the last 3 generations, POAG was found in 20 alive individuals. Most of the 20 patients had been diagnosed as having POAG before our examination. Two of the affected members are dead, but reliable ophthalmologic records could be examined. Two young persons in family A (IV4 and IV5, ages 20 and 18 years, respectively), who were considered unaffected on the basis of the clinical variables evaluated in the present study, were found to be affected on the basis of the genetic investigation because they harbored the same molecular defect found in patients with glaucoma. After these results became available, these 2 individuals were extensively assessed clinically, but no signs of glaucoma are as yet detectable. The Table shows the age at onset, intraocular pressure, and vertical cup-disc ratio found in this cohort of patients. Most of them (16 of 20) were diagnosed as having glaucoma at younger than 40 years (mean age, 25.8 years); only 1 patient from each family had a later onset (>40 years).

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.

#### 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 D1S242. In all 4 families, POAG was found in 20 alive individuals. Most of the 20 patients had been diagnosed as having POAG before our examination. Two of the affected members are dead, but reliable ophthalmologic records could be examined. Two young persons in family A (IV4 and IV5, ages 20 and 18 years, respectively), who were considered unaffected on the basis of the clinical variables evaluated in the present study, were found to be affected on the basis of the genetic investigation because they harbored the same molecular defect found in patients with glaucoma. After these results became available, these 2 individuals were extensively assessed clinically, but no signs of glaucoma are as yet detectable. The Table shows the age at onset, intraocular pressure, and vertical cup-disc ratio found in this cohort of patients. Most of them (16 of 20) were diagnosed as having glaucoma at younger than 40 years (mean age, 25.8 years); only 1 patient from each family had a later onset (>40 years).

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#### 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 unaffected 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.

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**Clinical Data of 4 Italian Families With Primary Open-angle Glaucoma (POAG)*

<table>
<thead>
<tr>
<th>Family</th>
<th>Patients With POAG, No.</th>
<th>Age at Diagnosis, y Range</th>
<th>Maximum Recorded IOP, mm Hg, Mean ± SD</th>
<th>Cup-Disc Ratio, Mean ± SD</th>
<th>Surgical Cases, No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7</td>
<td>14-48</td>
<td>40.6 ± 10.2</td>
<td>0.7 ± 2.0</td>
<td>7</td>
</tr>
<tr>
<td>B</td>
<td>5</td>
<td>14-54</td>
<td>39.6 ± 9.6</td>
<td>0.8 ± 2.0</td>
<td>5</td>
</tr>
<tr>
<td>C</td>
<td>5</td>
<td>25-43</td>
<td>36.9 ± 9.8</td>
<td>0.7 ± 2.0</td>
<td>3</td>
</tr>
<tr>
<td>D</td>
<td>3</td>
<td>8-44</td>
<td>32.3 ± 13.6</td>
<td>0.7 ± 2.0</td>
<td>1</td>
</tr>
<tr>
<td>All families</td>
<td>20</td>
<td>8-54</td>
<td>38.1 ± 10.0</td>
<td>0.7 ± 2.0</td>
<td>16</td>
</tr>
</tbody>
</table>

* IOP indicates intraocular pressure. All patients had a grade 3 or 4 lesion on gonioscopy, and all had abnormal visual fields.
We found an association between the 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 GLC1A gene allowed the identification of a novel mutation (1177GACA→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 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 θ=0.0).

The GLC1A gene consists of 3 exons that codify for a 504–amino acid glycoprotein, mainly expressed in the ciliary body, sclera, and trabecular meshwork. 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. 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. 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 GLC1A gene are missense located in the third exon. They may act as a dominant negative mutation leading to MYOC protein accumulation in the extracellular matrix and consequent outflow resistance. Support of this hypothesis will derive from more functional studies.

The 1177GACA→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. 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 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.
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