Myocilin Gln368stop Mutation and Advanced Age as Risk Factors for Late-Onset Primary Open-Angle Glaucoma

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Background: Juvenile open-angle glaucoma has been found to be associated with molecular defects in the myocilin (MYOC) gene. Most of the defects are missense mutations located in the third exon. The Gln368stop mutation has recently been found in several cases of late-onset primary open-angle glaucoma (POAG).

Objective: To study the effect of glaucoma risk in a relatively homogeneous genetic population.

Methods: A clinical study was performed in all living members of a 5-generation family. DNA analysis was performed for studying association with genetic markers and identifying the mutation.

Results: We identified the Gln368stop molecular defect in 19 patients with POAG, 5 patients with ocular hypertension, and 22 healthy carriers. We compared affected and unaffected carriers based on age at onset and last examination, respectively. Besides the presence of 3 young patients with POAG (<40 years old), the number of glaucomatous patients in the advanced age group increased.

Conclusions: The penetrance of glaucoma increases with age in Gln368stop carriers, but some remain unaffected at advanced age and others are affected at an early age. This suggests that additional risk factors are operating within this family, which may be identified by a genome-wide linkage search in this large pedigree.

Clinical Relevance: The myocilin Gln368stop mutation shows a good genotype-phenotype correlation and should be investigated in all familiar cases of chronic POAG. This may be important for early diagnosis and periodical checkups of presymptomatic individuals belonging to these families.


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PATIENTS AND METHODS

CLINICAL STUDIES

The family described in this study comes from Soave, a small town in the Veneto region in northeastern Italy. We collected information on 170 members (93 men, 75 women). Ten were already dead when the research started. Indirect clinical information was obtained for 46 members because some were dead and others unavailable. The remaining 124 members underwent a clinical examination that included case history, refraction analysis and visual acuity measurement, slitlamp analysis of the anterior segment, indirect binocular ophthalmoscopy, biomicroscopic analysis of the optic disc through a 78 diopter (D) lens, Goldmann application tonometry, threshold computerized perimetry using the Humphrey 640 perimeter Statpac 30-2 program (Zeiss, San Landro, Calif), and gonioscopy.

The diagnosis of POAG was based on the observation of at least 2 of the following abnormalities: glaucomatous optic disc damage, glaucomatous visual field defects, and high intraocular pressure (>21 mm Hg) in the presence of a normal open anterior chamber angle. Abnormalities of the optic disc are defined as follows: (1) concentric excavation with a vertical cup-disc (c/d) ratio of 0.7 or more; (2) oval excavation with a difference of more than 0.2 between vertical and horizontal c/d ratio; (3) notching of the neutral rim; (4) excavation reaching the disc margin; (5) disc hemorrhage; and (6) asymmetric disc excavation with a difference in vertical c/d ratio of more than 0.2 between the 2 eyes. The diagnosis of a glaucomatous visual field defect was formulated on the basis of the single field analysis characteristics with the use of the Humphrey Statpac II program and required agreement between the assessments of 2 perimetry experts. The perimetric defect type and stage evaluation was performed using the Brusini Glaucoma Staging System.\textsuperscript{20,21} The Brusini Glaucoma Staging System is a graphic method using a special diagram that classifies the visual field defects into 5 stages of severity and assigns them to 1 of 3 types—generalized, mixed, and localized—on the basis of the mean deviation and corrected pattern standard deviation values. Informed consent was obtained from all subjects participating in the study.

DNA ANALYSIS

DNA of 19 glaucomatous patients, 7 patients with OH, and 98 healthy individuals was extracted from peripheral blood cells using standard methods. We analyzed highly polymorphic microsatellite markers centromeric (D1S196) and telomeric (D1S218, D1S218) from the GLC1A locus. Polymerase chain reaction, involving incorporation of deoxyinosine 32-labeled adenosine triphosphate, was performed according to standard procedures. Primer sequences were obtained from the Genome Data Base. For allele scoring, 2 µL of polymerase chain reaction products were size fractionated on 6% polyacrylamide gel and autoradiographed. Sequence analysis of the MYOC gene coding region was obtained according to Adam et al.\textsuperscript{9}

To screen for the presence of the C\textrightharpoonup T substitution, we performed dot blot analysis on amplified DNA as previously described,\textsuperscript{22} with 2 allele-specific oligonucleotide probes, one complementary to the Gln368stop mutation and the other homologous to normal DNA at the same position. The allele-specific oligonucleotide probe sequences were the following: normal, CAC GGA CAG TTC CCG, and mutated, CAC GGA TAG TTC CCG.

STATISTICAL ANALYSIS

Two-point lod scores were calculated by the MLINK and LINK routines of FASTLINK\textsuperscript{23,24} package version 4.0P (Rice University, Houston, Tex). The disease-allele frequency was 0.0001, considering marker-allele frequencies to be equal to each other. Age-dependent penetrance classes were constructed based on the age at diagnosis of the family patients. We specified 5 liability classes, in which penetrance increased from 0 at age 35 years to 0.75 at age 70 years, after which it remained constant at 0.75.

Support for linkage was evaluated by multipoint lod score analysis\textsuperscript{25} by use of the LINKMAP routine of FASTLINK. We analyzed 3 markers (D1S196, D1S218, D1S218) that span approximately 10.3 cM on the 1q21-31 region on chromosome 1. The references genetic map used for linkage analysis was obtained from the Genome Data Base.
All the patients with glaucoma had fairly homogeneous phenotypic characteristics (Table 1).

The mean±SD age was 75.2 ± 10.3 years (range, 48-86 years). The mean±SD age at onset was 56.3 ± 10.9 years. Three patients were younger than 40 years, but none were younger than 36 years.

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PHENOTYPE OF PATIENTS WITH GLAUCOMA

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Optic disc damage was extremely variable and difficult to quantify in detail.

Pedigree of a family with a large number of members with primary open-angle glaucoma. Roman numerals indicate generations; squares and circles, men and women, respectively; black symbols, individuals affected by glaucoma; blue symbols, individuals affected by ocular hypertension; open symbols, healthy individuals; slashed symbols, deceased members; arrows, affected individuals who do not have the Gln368stop mutation; and dotted symbols, asymptomatic carriers.
The vertical c/d ratio was less than 0.5 in 6 eyes, between 0.5 and 0.7 in 15 eyes, and greater than 0.7 in another 11 eyes. Among the latter, there were only 4 eyes with total excavation. Data are lacking for 6 additional eyes. In one of them, vision was completely absent.

Visual field status was also variable. The damage was severe in 10 eyes (7 of them were blind). Six eyes had medium to severe damage (Brusini grades 3-4), whereas in 11 eyes damage was slight (Brusini grades 1-2). The visual field was normal in 5 eyes.

The eyes with loss of visual function belonged to people older than 80 years. In contrast, our cases were very homogeneous from the gonioscopic point of view. Of 18 patients for whom data were available, 16 showed a wide unoccludable angle with normal morphologic structure, 1 had a bilateral wide angle with the presence of iridial processes, and 1 had a narrow angle with the presence of few goniosynechiae apparently due to previous episodes of angle closure. The investigators believed that this represented a case of combined mechanism glaucoma. Refraction data were available for 31 eyes: 12 of them were emmetropic, 4 hyperopic, 2 astigmatic, and 13 myopic. Of the myopic eyes, 2 belonged to the same patient and had a high degree of myopia (−7 D). Best-corrected visual acuity varied remarkably among patients, but the variability was influenced by the presence of cataracts or senile retinal changes. Only 8 eyes could be considered blind as a result of glaucoma and belonged to patients whose average age was 83 years. In one of them, vision was completely absent.

We performed linkage analysis with genetic markers on chromosome 1q21-31, closely linked to the GLCIA locus in all patients recruited into the clinical study. We identified a haplotype, which segregates with the disease in all branches of the family. This haplotype is present in all 19 glaucomatous patients, all but 2 of the 7 hypertensive patients, and several young unaffected individuals. The maximum pairwise lod score was obtained with D1S2815 (maximum Zmax=3.18 at θ=0.0). The multipoint analysis with all the markers resulted in an increased evidence of linkage with a peak multipoint lod score of 4.87.

Direct sequencing of the MYOC gene coding region in 2 of the patients revealed a C→T transition at the first base position of codon 368, which results in a substitution of a stop codon for a glutamine (Gln368stop).6 Screening of the family with allele-specific oligonucleotide probes showed segregation of the mutation with the disease. Forty-six members of the family had the mutation, 19 of whom had glaucoma and 5 of whom had OH. In 2 patients with OH, related by ancestry, mutation was absent (Figure 1; IV:19, IV:87). In one case, the disease was probably inherited from the mother (Figure 1; III:43, IV:19) and involved a different POAG gene.

Of the 46 individuals who had the mutation, only 24 (52%) were found to have POAG or OH.

### Table 2. Clinical Characteristics of Patients With Ocular Hypertension*

<table>
<thead>
<tr>
<th>Siblings</th>
<th>Age, y</th>
<th>Age at Diagnosis, y</th>
<th>Maximum Recorded IOP, OD/OS</th>
<th>Vertical Cup-Disc Ratio, OD/OS</th>
<th>Visual Field Status†</th>
<th>Angle‡</th>
<th>Refraction</th>
<th>Visual Acuity</th>
<th>Therapy§</th>
<th>Gln368stop Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>III:27</td>
<td>67</td>
<td>60</td>
<td>29-29</td>
<td>N-N</td>
<td>N-N</td>
<td>3-4</td>
<td>3-4</td>
<td>emm</td>
<td>10/10</td>
<td>P2</td>
</tr>
<tr>
<td>IV:19</td>
<td>45</td>
<td>43</td>
<td>25-26</td>
<td>N-N</td>
<td>N-N</td>
<td>3-4</td>
<td>3-4</td>
<td>emm</td>
<td>10/10</td>
<td>P2</td>
</tr>
<tr>
<td>IV:32</td>
<td>57</td>
<td>48</td>
<td>22-22</td>
<td>N-N</td>
<td>N-N</td>
<td>3-4</td>
<td>3-4</td>
<td>emm</td>
<td>10/10</td>
<td>P2</td>
</tr>
<tr>
<td>IV:43</td>
<td>50</td>
<td>46</td>
<td>25-25</td>
<td>N-N</td>
<td>N-N</td>
<td>2-2</td>
<td>2-2</td>
<td>emm</td>
<td>10/10</td>
<td>P1</td>
</tr>
<tr>
<td>IV:79</td>
<td>51</td>
<td>48</td>
<td>24-24</td>
<td>N-N</td>
<td>N-N</td>
<td>3-4</td>
<td>3-4</td>
<td>emm</td>
<td>10/10</td>
<td>P1</td>
</tr>
<tr>
<td>IV:87</td>
<td>58</td>
<td>?</td>
<td>26-26</td>
<td>N-N</td>
<td>N-N</td>
<td>3-4</td>
<td>3-4</td>
<td>emm</td>
<td>10/10</td>
<td>P1</td>
</tr>
<tr>
<td>IV:91</td>
<td>37</td>
<td>28</td>
<td>23-24</td>
<td>N-N</td>
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<td>3-4</td>
<td>3-4</td>
<td>emm</td>
<td>10/10</td>
<td>Present</td>
</tr>
</tbody>
</table>

Mean ± SD 52.14 ± 9.70 45.50 ± 10.35 24.86 ± 2.27

* IOP indicates intraocular pressure; N, normal; ellipses, data not applicable; and emm, emmetropic.
† Visual field Brusini classification.
‡ Chamber angle, Shaffer classification.
§ P1 indicates 1 pharmacologic treatment; P2, 2 pharmacologic treatments.

### GENETIC ANALYSIS

We identified a large multigenerational family with several cases of POAG. Our study was conducted in a...
small town in Italy that had a highly inbred population because of geographic isolation. This genetically homogeneous population is ideal for the study of complex diseases such as glaucoma. The phenotype of this POAG family is characterized by adult age of onset and moderately elevated intraocular pressure, which is well controlled by medical therapy and tends to cause blindness only in old age. Mild ocular hypertension was present in several individuals. The association of the GLC1A locus with the disease in this family was ascertained by linkage analysis of several affected family members. Sequence analysis of the GLC1A gene revealed a Gln368stop mutation in all glaucomatous patients (n=19). In addition, all but 2 (IV:19 and IV:87) of the patients with OH (n=7) had the mutation. Individual IV:87 probably inherited the phenotype from the hypertensive mother, who was not related to the family. The OH of patient IV:19 may be due to other factors unrelated to chromosome 1. In addition, 22 healthy individuals were found to be carriers of the same mutation.

The MYOC gene codes for a glycoprotein called myocilin. The ocular expression of this gene is primarily found in the ciliary body, sclera, and trabecular meshwork. Analysis of myocilin amino acid sequence defines a signal peptide at the N-terminus: a leucine zipper motif at every 7th position and 5 arginine residues at every 11th position, which are 25% homologous to the myosin heavy chain. This suggests that myocilin is a membrane-associated protein that causes protein-protein interactions. The C-terminus, coded for the third exon, shares significant homology with several olfactomedin-like glycoproteins. This domain may be important for protein uptake and metabolism by the trabecular meshwork cells. To date, most of the POAG mutations identified in myocilin are missense mutations located in the third exon associated with JOAG.

Alward et al and Allingham et al have described the Gln368stop mutation associated with late-onset POAG in several families. In these 2 studies, the mean age of diagnosis was 59 years in 15 families and 61.8 years in 3 families, respectively. However, in the study by Alward et al, several glaucomatous patients within the same family did not carry the mutation. Their reports do not specify the severity of the Gln368stop mutation compared with the more severe phenotype produced by missense mutations or the role played by this molecular defect in the pathogenesis of the disease.

We analyzed the large cohort of Gln368stop carriers in age groups based on age at diagnosis for patients with POAG and OH and the age at the last examination for healthy patients. The mean age of healthy carriers is 48.2 years, and the mean ages at diagnosis of patients with OH and patients with glaucoma are 45.5 and 56.3 years, respectively. It is possible, therefore, that some of the healthy mutation carriers may develop glaucoma in the future. We found that 54% of people who carry the Gln368stop mutation have already developed OH or POAG. We subdivided these carriers into 4 categories by age as shown in Table 3. In the younger than 40 years and 41- to 50-year age groups, the percentage of healthy carriers is similar to the percentage of patients with OH and POAG. In the 51- to 60-year age group, the number of patients with POAG increases. In the highest age group (61-70 years), 72.7% have glaucoma, but 27.3% are still unaffected, with an identical mean age.

Our data suggest that both the Gln368stop mutation and the presence of advanced age are important risk factors for the development of the disease in this family. These 2 risk factors, however, are not always related to one another, as demonstrated by the 3 young patients with POAG in the family. Moreover, the presence of 3 healthy carriers in the oldest age group suggests that these 2 risk factors alone are not sufficient for development of the disease. The large number of healthy carriers in the same age group as our cohort of patients suggests that other genetic and/or nongenetic factors are needed for development of the disease.

The Gln368stop mutation was not found in several families with a similar POAG phenotype from the same village (data not shown). This result was unexpected, since several studies of JOAG demonstrate a founder mutation in different closed communities. Our findings support the hypothesis that additional genetic factors associated with POAG are present in this village. Obviously, we cannot exclude the presence of environmental factors that conferred an elevated risk of the disease.

Several loci have already been found to be associated with adult-type POAG, namely GLC1B (2cen-q13), GLC1C (3q21-24), and GLC1D (8q23). Linkage analysis with markers linked to these loci excluded any association in our family (data not shown). The large number of patients and healthy carriers, all within the same family, and the finding of 2 independent risk factors may facilitate the identification of additional genetic factors by a genome-wide search.

| Table 3. Number of Cases and Age at Diagnosis of Gln368stop Carriers* |
|---------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                                | <40            | 41-50          | 51-60          | 61-70          | <40            | 41-50          | 51-60          | 61-70          |
| No. (%) of Cases               | No. (%) of Cases | Mean Age, y | No. (%) of Cases | Mean Age, y | No. (%) of Cases | Mean Age, y | No. (%) of Cases | Mean Age, y |
| Healthy carriers              | 7 (64)        | 34            | 7 (58)        | 45.7          | 5 (42)        | 53            | 3 (27)        | 65           |
| Patients with POAG            | 3 (27)        | 37.3          | 2 (17)        | 44.5          | 6 (50)        | 59            | 8 (73)        | 65.25        |
| Patients with OH              | 1 (9)         | 28            | 3 (25)        | 47.3          | 1 (8)         | 60            | 0             | ...          |

*Mean age is at time of diagnosis for patients with primary open-angle glaucoma (POAG) and ocular hypertension (OH) and last examination for healthy carriers.
REFERENCES


