CYP1B1 and MYOC Mutations in 116 Chinese Patients With Primary Congenital Glaucoma

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Objectives: To clinically characterize 116 Chinese patients with primary congenital glaucoma and to determine the role of CYP1B1 and MYOC mutations in this cohort.

Methods: This study included 116 unrelated patients with primary congenital glaucoma and 120 ethnically matched, unrelated, healthy controls in China. CYP1B1 and MYOC were amplified from genomic DNA, followed by direct DNA sequencing to identify disease-causing variants.

Results: Twenty patients (17.2%) had CYP1B1 mutations. Five of these patients had homozygous mutant alleles and 4 had compound heterozygous mutations. Fourteen of the mutations were novel. Three patients (2.6%) had MYOC mutations, all of which were novel.

Conclusions: This study describes the spectrum of CYP1B1 and MYOC mutations in a large cohort of Chinese patients with primary congenital glaucoma. The role of mutations in CYP1B1 and MYOC varies, depending on the ethnic origin of the patients.

Clinical Relevance: Patients with primary congenital glaucoma and CYP1B1 mutations tend to have a more severe phenotype than those without mutations. Genetic testing of CYP1B1 mutations may help predict new cases and their prognoses.

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Primary congenital glaucoma (PCG), caused by developmental defects in the anterior chamber angle, usually has its onset in the neonatal or infantile period. Left untreated, PCG may result in optic atrophy and permanent vision loss. The incidence, which varies considerably in different geographic locations and in different ethnic groups, is approximately 1 in 10,000 births. A greater incidence occurs in populations with higher rates of consanguinity (eg, 1 in 1250 in Romanian gypsies,1 in 2500 in Saudi Arabia,2 and 1 in 3300 in Andhra Pradesh, India3). Most cases of PCG are sporadic, but 10% to 40% are familial. Most familial cases are associated with consanguinity and are characterized by an autosomal recessive mode of inheritance with variable penetrance.4,5

Primary congenital glaucoma seems to be a genetically heterogeneous disorder.6-10 Three loci for PCG have been mapped: GLC3A (2p21),7 GLC3B (1p36),8 and GLC3C (14q24.3).9 Of these 3 loci, only 1 gene, CYP1B1, a member of the P450 superfamily,10 in locus GLC3A has been identified. More than 60 mutations in CYP1B1 have been described (http://www.hgmd.cf.ac.uk/ac/gene.php?gene=CYP1B1). Although more commonly associated with open-angle glaucoma, mutations in the myocilin gene (MYOC) (http://www.myocilin.com)11 have been identified in approximately 5.5% of cases of PCG.12 Some patients with PCG exhibit both MYOC and CYP1B1 mutations.13

In this study, we clinically characterized 116 Chinese patients with PCG. We sequenced the CYP1B1 and MYOC genes in each patient to describe the spectrum of CYP1B1 and MYOC mutations in Chinese patients with PCG.

Methods

Patients

The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the ethics committee of the Shanghai Medical School, Fudan University. Written informed consent was obtained from all the patients or, for children, from their parents.

All the patients underwent a complete ocular examination, including slitlamp biomicroscopy, ophtalmoscopy, applanation tonometry, and gonioscopy, when available. Patients with other recognized associations or combinations of infantile glaucoma, such as aniridia, anterior segment dysgenesis, congeni-
tal hereditary endothelial dystrophy, neurofibromatosis, and Sturge-Weber syndrome, were excluded. The affected status was defined by an intraocular pressure higher than 21 mm Hg and by the presence of corneal enlargement, a scar, or Haab striae. A total of 116 patients were included in the study, and 120 healthy individuals with normal ophthalmic examination findings and no reported family history of hereditary eye diseases were recruited as controls.

NUCLEOTIDE SEQUENCE ANALYSIS

Genomic DNA was prepared from peripheral blood using the QIAmp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany). The coding regions (exons 2 and 3) and the promoter region of CYP1B1 (GenBank U56438) and the coding regions of MYOC (GenBank AF001620) were amplified from genomic DNA by means of polymerase chain reaction (eTable 1; http://www.archophthalmol.com).

Polymerase chain reaction amplification was performed in a 25-µL mixture containing genomic DNA, 50 ng; forward and reverse primers, 0.5 µmol/L; magnesium chloride, 1.5 mmol/L; 5% dimethylsulfoxide (only for CYP1B1_promoter, CYP1B1_2A, CYP1B1_2B, and CYP1B1_2C); dNTP, 0.2 µmol/mL of each; and Taq polymerase, 1.25 U (Takara Bio Inc, Shiga, Japan). Polymerase chain reaction conditions were as follows: an initial denaturation at 95°C for 5 minutes followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at a temperature specific for each primer for 45 seconds, and extension at 72°C for 1 minute. A final extension at 72°C for 10 minutes completed the reaction. Thermocycling was performed on a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, California). Direct sequencing with forward and reverse amplification primers was performed using an Applied Biosystems 3730 DNA Analyzer, using the BigDye Terminator v3.1 protocol.

RESULTS

All the patients were diagnosed as having PCG before age 3 years. The median age at diagnosis was 4 months. A family history of PCG could be established for only 2 patients. A history of consanguinity could be established
for only 1 patient. There were 87 male patients and 29 female patients, a male to female ratio of approximately 3:1. There were 79 bilaterally affected individuals and 37 unilaterally affected individuals, a ratio of more than 2:1.

Sequence analysis of the coding regions of the CYP1B1 gene in the 116 patients with PCG revealed 20 different variants that were not detected in the 120 control subjects (Figure 1). Of the 20 patients with CYP1B1 mutations, 5 were homozygotes, 4 were compound heterozygotes, and 11 were heterozygotes. Of these 20 variants, 14 are reported for the first time and 6 were reported in previous studies (Table 1). Of the 14 novel mutations, the single nucleotide deletion g.3972delC (p.A56GGH) resulted in a premature stop codon at the 59th amino acid, the mutation g.4168_4169insGACCGGCGCGCTTCGCC (p.A121_S122insDRPAFA) inserted 6 amino acids, and g.8209_8213delAGCAGinsTTGTTGAAAAA (C).

In agreement with previous studies, our study patients with CYP1B1 mutations tended to have PCG at an earlier age and were more likely to have bilateral disease compared with patients with PCG without CYP1B1 mutations. The median age at diagnosis for CYP1B1 mutation carriers (1.5 months) was significantly lower than that for patients without CYP1B1 mutations (6.0 months) (z = 2.72, P = .007, Wilcoxon-Mann-Whitney test). Of the patients with CYP1B1 mutations, 85% had bilateral disease compared with 65% of those without mutations (χ² = 3.18, P = .08, Pearson χ² test) (eTable 2).

Haplotypes were deduced on 8 common variants in CYP1B1 (g.2805C>T, g.3130C>T, g.3793T>C, g.3947C>G, g.4160G>T, g.8131C>G, g.8184C>T, and g.8195A>G) and were compared between patients and controls using the PHASE v2.0 program. The most common haplotypes, in order, in these patients and controls were 5’-CCCCGCCA-3’, 5’-TCTGTCCA-3’, and 5’-CCCGGTA-3’ (eTable 3). The most predominant haplotype in the present study is different from that in most previous studies in the Western world but is similar to that found in a Japanese study (Table 2).
Three novel MYOC mutations were observed in 3 patients without CYP1B1 mutations that were not observed in any of the 120 controls. These mutations—c.688G>A (p.E230K), c.814C>T (p.R272X), and c.938C>T (p.S313F)—changed conserved amino acids (eFigure). In total, 93 of 116 patients had neither CYP1B1 mutations nor MYOC mutations, and no one had a mutation in both genes.

Mutations in CYP1B1 have been associated with PCG with varying frequencies across different ethnic communities and geographic boundaries. In this study, 20 of 116 patients with PCG (17.2%) had CYP1B1 mutations. This percentage is much lower than the 90% to 100% reported in Arab patients with PCG and in Romany patients with PCG, and it is also lower than the 30% to 50% reported in Indian and Brazilian patients with PCG. The percentage is similar to that detected in a study of Japanese patients with sporadic PCG. Because of low rates of inbreeding and consanguinity in our study patients with PCG, these results are more comparable with the study of Japanese patients with sporadic PCG.

Primary congenital glaucoma is generally inherited in an autosomal recessive pattern. Mutations are usually homozygous or compound heterozygous in affected individuals and with typically normal heterozygotes, whereas in this study, 11 patients were heterozygotes of CYP1B1. In fact, heterozygous mutations of CYP1B1 in patients with PCG have been previously reported. It was reasonable to assume that undetected variations (eg, in the promoter region) affected gene function or expression. However, in our study patients, no mutation was found in the promoter region of the gene. The coding regions of MYOC were also sequenced because previous studies showed a possible role of MYOC mutations in PCG. However, MYOC was involved in only 3 of the 116 patients (2.6%) with PCG; these 3 patients carried no CYP1B1 mutations. None of our study patients had a mutation in both CYP1B1 and MYOC. It seems that there are other genes involved in the pathogenesis of PCG.

In the CYP1B1 gene, a transmembrane domain is present at the N-terminal, followed by a proline-rich "hinge" region that permits flexibility between the membrane-spanning domain and the cytoplasmic portion of the molecule. A set of conserved structure elements is present at the C-terminal. Substrate recognition site regions play important roles in substrate binding. The homozygous deletion at g.3972delC resulted in truncation of the CYP1B1 protein to 58 amino acids, thereby deleting 485 amino acids from the C-terminus of the protein. This deletion occurred at the end of the membrane domain, resulting in the elimination of all-important domains in exons 2 and 3. The mutation p.A121_S122insDRAFA was located in substrate recognition site 1, and the mutation p.S438_R459delinsLKK occurred at the end of the meander region, which precedes the heme-binding region and could theoretically affect the configuration of the region. Most of the missense mutations were located in conserved regions, such as the hinge region (160M), E-helix

### Table 2: Predominant Haplotypes in Patients With Primary Congenital Glaucoma in Different Nations

<table>
<thead>
<tr>
<th>Single-Nucleotide Polymorphisms</th>
<th>g.2805</th>
<th>g.3130</th>
<th>g.3793</th>
<th>g.3947</th>
<th>g.4160</th>
<th>g.8131</th>
<th>g.8184</th>
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<td>cyp1b1-human</td>
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*Polymermorphisms are shown from the 5' end to the 3' end. Blank cells indicate the absence of the specific polymorphisms.*
(N203S and S215I), I-helix (N319S), J-helix (V363D and V364M), K-helix (R390H), meander region (R444Q), heme-binding region (R469W), and substrate recognition site region (P1185 and F1345) (Figure 1). Other new missense mutations (V95A, L107V, P1185, F1345, N203S, A287S, D291G, and R368L) did not occur in conserved regions, but comparison of amino acid sequence alignment in different cytochrome P450 proteins revealed that these missense mutations altered evolutionarily conserved amino acids (Figure 3).

A wide range of mutations in the coding regions of CYP1B1 were detected in these patients. Most of these mutations were sporadic and have not been previously reported in studies of other ethnic groups. No common variants were found, such as E387K observed in Slovakian Romany people,21; G61E, D374N, and R469W described in a Saudi Arabian population; and the predominant R368H seen in Indian patients.22 The different mutation spectrum in our study patients may be attributed to genetic heterogeneity and ethnic diversity. Different haplotype backgrounds may, in part, explain the striking dissimilarities in mutations observed in this study compared with those observed in studies of other ethnic groups (Table 2).22

In summary, the present study describes the spectrum of CYP1B1 and MYOC mutations associated with PCG in a Chinese population. A different pattern of CYP1B1 sequence variations seems to exist in Chinese patients compared with patients from other ethnic backgrounds. Mutations of the CYP1B1 gene are much less prevalent in Chinese populations with PCG compared with Arabic or Romany populations, and MYOC mutations play a minor role in PCG. A better understanding of the genetics of PCG will lead to earlier diagnosis and better treatment of this severe, blinding condition.

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REFERENCES


