Molecular analysis of Italian patients affected by Primary Congenital Glaucoma

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Abstract

PURPOSE. To determine the possible genetic defect underlying Primary Congenital Glaucoma (PCG) in Italy, molecular analysis was performed on CYP1B1 and MYOC/TIGR genes.

METHODS. Blood samples from 72 patients with a PCG diagnosis including high intraocular pressure (IOP), epiphora, corneal edema, photophobia, blepharospasm and ocular enlargement were collected to perform DNA extraction and sequence analysis.

RESULTS Seventeen different variations of CYP1B1 were found in 25 of the 72 (34.7%) PCG patients. Twelve of these changes had been identified in previous reports as disease-causing mutations, while L26R, P52L, A106D, A237E and F440L are described here for the first time. The F440L has always been found in cis with the P52L, both in patients and in healthy carriers, suggesting its role as a rare polymorphism linked to the P52L; the other new found variations could possibly play a pathogenetic role. G61E and 1775-1801dup27 are the most frequent mutations in our patients. Previously described CYP1B1 polymorphisms (R48G, A119S, L432V, D449D and N453S) were also analysed and a particular haplotype was identified in affected individuals with CYP1B1 mutations.

The transcript region of the MYOC/TIGR gene was studied in all the patients bearing only one mutation in CYP1B1 gene, and two aminoacidic variations (A447V and R76K) were identified.
CONCLUSIONS. Our results confirm the major role of CYP1B1 gene in Italian congenital glaucoma and also propose an autosomic recessive role of MYOC/TIGR in a digenic inheritance model.
Introduction

Glaucoma is a heterogeneous group of optic neuropathies, which can lead to optic nerve atrophy and permanent loss of vision. It is the second most prevalent cause of bilateral blindness in the Western World and it affects several million people worldwide. There are different types of glaucoma, more often with multifactorial aetiology (1). Glaucoma could be defined as primary open-angle glaucoma of adult onset, the commonest form of the disease, representing almost half of all cases; as juvenile-onset open-angle glaucoma, whose manifestation start predominantly before the fourth decade of life (2), and as primary congenital glaucoma (PCG) a clinical and genetic entity distinct from the juvenile form.

PCG may be associated with ocular malformation, such as aniridia (3) or congenital hydrocephalus and anterior segment dysgenesis (4). It results from poorly understood developmental abnormalities of the ocular drainage structures and is clinically characterised by high intraocular pressure (IOP), epiphora, corneal edema, photophobia, blepharospasm and ocular enlargement (5). The disease is bilateral in approximately 75% of cases. PCG incidence varies substantially among countries: it is estimated to occur in 1/10,000 births in Europe, in 1/2500 in Saudi Arabia and 1/1250 in the gypsy population of Slovakia. (Online Mendelian Inheritance in Man, OMIM 231300).

An increase in IOP, untreated with surgery or pharmacological therapy, results in ocular enlargement (buphthalmos) and rapidly progressive cupping of the optic nerve with severe and irreversible alteration of the visual field (6). Early recognition and appropriate therapy can significantly improve the child's visual future. Onset of an aggressive form of glaucoma occurs between 0 and 3 years of age. The disease has higher prevalence in males (65% of cases).

Most cases of PCG are sporadic and recessive inheritance of PCG is common, with almost
complete penetrance in populations with a high consanguinity rate. Reduced penetrance (40% in some populations) and various phenotypic forms suggests a polygenic inheritance pattern or multifactorial aetiologies.

Genetic linkage studies, started in 1995, identified two separate loci (GLC3A on 2p21 and GLC3B on 1p36) associated with the disease, thus confirming that PCG is genetically heterogeneous (7, 8, 9). A few years later Stoilov reported that the cytochrome P4501B1 gene (CYP1B1, OMIM 601771), located within the GLC3A locus, was mutated in individuals with PCG (10).

CYP1B1 belongs to a multigene superfamily of monomeric mixed-function monooxygenases, responsible for the phase I metabolism of a wide range of structurally different substrates including steroids and retinoids (11,12). A specific CYP1B1 metabolite is most probably required for normal eye development, and its deficiency (or toxic accumulation) may result in PCG (13).

The CYP1B1 gene maps to 2p22-p21, contains 3 exons and 2 introns with a putative Open Reading Frame that starts in the second exon, and is expressed in many normal human tissues (14). Northern hybridisation analysis showed strong expression of CYP1B1 in the anterior uveal tract, which is involved in secretion of the aqueous humour and in the regulation of outflow facility, processes that could contribute to the elevated intraocular pressure characteristic of PCG (15).

An extensive allelic heterogeneity was illustrated by a comprehensive sequence analysis of the translated regions of the CYP1B1 gene in 22 PCG families and in 100 randomly selected normal individuals. Sixteen mutations and 6 polymorphisms were identified. The positions affected by these changes were evaluated by building a 3-dimensional homology model of the conserved C-terminal half of CYP1B1. These mutations may interfere with the heme
incorporation by affecting the hinge region and/or the conserved core structures (CCS) that determine the proper folding and heme-binding ability of P450 molecules. In contrast, all polymorphic sites were poorly conserved and located outside the CCS (15).

More than 50 CYP1B1 pathogenic mutations, including 34 missense/nonsense substitutions, 10 small deletions, 6 small insertions, a 27 Bp duplication and a deletion involving part of intron 2 and exon 3, have been described in various ethnic groups (Human Gene Mutations Database). Two CYP1B1 mutations, Met1Thr and Trp57Stop, in a compound heterozygosis status, have been identified as the molecular basis of Peters’ anomaly that consists of corneal opacity, defects in the posterior structures of the cornea, iridocorneal and/or keratolenticular adhesions (16).

Mutations in CYP1B1 were found also in patients with juvenile glaucoma (JOAG), an early-onset form of open angle glaucoma that can be caused by a mutation in the gene encoding myocilin (MYOC/TIGR). Vincent et al. studied the role of the CYP1B1 and MYOC/TIGR and found that MYOC/ TIGR mutations included cases of juvenile glaucoma (with or without pigmentary glaucoma) and mixed-mechanism glaucoma, and CYP1B1 mutations involved cases of juvenile open angle glaucoma as well as cases of congenital glaucoma. This work emphasised the genetic heterogeneity of juvenile and congenital glaucoma and demonstrated that the spectrum of expression of MYOC/ TIGR and CYP1B1 mutations is greater than expected. It also appeared that CYP1B1 may act as a modifier of MYOC/ TIGR expression and that these 2 genes may interact through a common pathway (17). Both CYP1B1 and the MYOC/TIGR genes are expressed in the iris, trabecular meshwork, and ciliary body of the eye (18).

Different CYP1B1 mutations together with a common MYOC/TIGR mutation (Gln48His) have been reported in PCG patients (19,20), thus suggesting a role for the MYOC gene in primary
congenital glaucoma via digenic interactions with CYP1B1.

In 22 Saudi Arabian families, 40 apparently unaffected individuals had mutations in CYP1B1 and haplotypes identical to their affected siblings. Analysis of these 22 relatives suggested the presence of a dominant modifier locus that is not linked genetically to CYP1B1. Linkage and Southern analyses excluded 3 candidate modifier loci, the arylhydrocarbon receptor (AHR) on 7p15, the arylhydrocarbon receptor nuclear translocator (ARNT) on 1q21, and the CYP2D6 gene on 22q13.1 (21).

Preliminary results on Italian population have been recently reported (Giuffrè I., “IOVS”, 2004, “Arvo E-Abstract”, 4398). To determine the possible role of the genetic defects so far described in PCG cases in Italy, molecular analysis of 72 patients was undertaken, and the CYP1B1 and MYOC genes were screened for mutations. We describe here the variations that we found in our population and the haplotype for five intragenic SNPs associated with the identified mutations.
Methods

Subjects
Blood samples were collected from 72 patients (10 subjects belonging to 5 families and 62 unrelated subjects) with a diagnosis of bilateral PCG at different hospitals in Northern, Central and Southern Italy. A clinical report with demographic data and clinical evaluation was obtained for every patient. Reports contain subjective symptoms (irritability, sensitivity to light, tearing of the eyes) and objective evaluation (corneal diameter enlargement, type of corneal edema, rupture of Descemet membrane, increase of IOP). Age of disease onset and number of surgery events are described in Table II.

Blood samples were also collected, when available, from parents. Informed consent to participate in this study was obtained from parents of every patient, both for themselves and for their children, using interviews and written informative material and forms approved by the Ethics Committee of Niguarda Ca’ Granda Hospital. The research followed the tenets of the Declaration of Helsinki.

Mutation Screening
DNA was extracted from peripheral blood of patients and family members.

The entire transcript region of the CYP1B1 gene, organised in three exons of which only II and III are translated, was screened for mutations using four sets of primers:

Set1 - cyp1b1Ex1F 5’-GCT TTG ACT CTG GAG TGG G-3’ and cyp1b1Ex1R 5’-TCC ATC TGA AGA GGT CGC C-3’ (424 Bp); set2 - cyp1b1Ex2aF 5’-TGA GTG TCA CGC CTT CTC C-3’ and cyp1b1Ex2aR 5’-CTC AGC ACG TGG CCC TC-3’ (548 Bp); set3 - cyp1b1Ex2bF 5’-ATG CGC AAC TTC TTC ACG-3’ and cyp1b1Ex2bR 5’-AGA GGA GAA AAG ACC TGG C-3’ (631
Bp); set4 - cyp1b1Ex3F 5'-TGC TCA CTT GCT TTT CTC TC-3' and cyp1b1Ex3R 5'-ATT TTA CTC CTC ATC TCC GAA-3' (691 Bp). PCR amplification was performed in a 50-µL volume consisting of 50 to 100 ng genomic DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM dNTPs, 0.8 microM of each primer and 1 U Taq polymerase (AmpliTaq Polimerase - Perkin Elmer) with 10% dimethyl sulfoxide (DMSO) only for set 2 and set 3. PCR amplification procedures were executed under the following conditions: for set 1 and set 4, 2 cycles consisting of 60 seconds at 94°C, 30 seconds at 52°C and 90 seconds at 72°C followed by 35 cycles each consisting of 30 seconds at 94°C, 30 seconds at 54°C and 90 seconds at 72°C, followed by a final extension of 7 minutes at 72°C; for set 2 and set 3, 12 cycles consisting of 60 seconds at 94°C, 120 seconds at 64°C with a decrement of 1°C every cycle and 180 seconds at 72°C followed by 25 cycles each consisting of 60 seconds at 94°C, 120 seconds at 52°C and 5 minutes at 72°C, followed by a final extension of 7 minutes at 72°C.

Patients with none or only one mutation in the CYP1B1 gene were screened for mutations in the transcript region of the MYOC/TIGR gene using three sets of primers:

Set 1 - myocEx1F 5'-GGC TGG CTC CCC AGT ATA TA- 3' and myoc1Ex1R 5'-CTG CTG AAC TCA GAG TCC CC- 3' (760 Bp); set 2 - myocEx2F 5'-AAC ATA GTC AAT CCT TGG GCC- 3' and myocEx2R 5'- TAA AGA CCA TGT GGG CAC AA- 3' (230 Bp); set 3 - myocEx3F 5'-TTA TGG ATT AAG TGG TGC TTC G- 3' and myocEx3R 5'- AGC ATC TCC TTC TGC CAT TG- 3' (870 Bp). PCR amplification was performed in a 25-µL volume consisting of 50 to 100 ng genomic DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM dNTPs, 0.8 microM of each primer and 1 U Taq polymerase (AmpliTaq Polimerase - Perkin Elmer). PCR amplification procedures were executed as follows: 35 cycles consisting of 60 seconds at 94°C, 60 seconds at 55°C and 120 seconds
at 72°C followed by a final extension of 7 minutes at 72°C.

PCR products were sequenced on an automated DNA sequencer (ABI-310, PE Applied Biosystems).

Every nucleotide change is indicated using the sequence of the cDNA published with the accession number NM_000104.2 for the CYP1B1 and NM_000261 for the MYOC/TIGR transcript. The position of mutations was described as in Recommendations for a Nomenclature System for Human Gene Mutations (22).

**Comparative Sequencing Alignment**

The cytochrome P450 1B1 and MYOC/TIGR sequences were obtained from GenBank provided by the National Centre for Biotechnology Information, Bethesda, MD (http://www.ncbi.nlm.nih.gov/Genbank).

Computer-assisted sequence alignment was performed with the pattern-induced multisequence alignment program (PIMA, provided by Baylor College of Medicine Search Launcher and available at: http://searchlauncher.bcm.tmc.edu).
Results

**CYP1B1 gene sequence analysis**

Direct sequencing of the three exons of *CYP1B1* gene was performed on 72 affected individuals belonging to 67 families from different Italian regions.

Twenty-five patients (34.7%) were carriers of mutations in the coding region of CYP1B1 gene: 17 were compound heterozygotes, 2 were homozygotes, and 6 carried only one mutation in a single allele (Table I).

The analysis was also performed on relatives of mutated subjects in order to verify the allele segregation and to complete the family study.

Of the seventeen different CYP1B1 variations identified, twelve had been previously characterised as pathogenetic: six aminoacid substitutions (M1T, G61E, R368H, E387K, R390S and A443G), two stop codons (W57X, R355X), two insertions (1236-1237insC and 1581-1582ins TCATGCCACC), one deletion (1436-1448delGAGTGCAGGCAGA) and one duplication (1775-1801 dupGGCGGTGCATTGGCGAAGAACTTTCTA). Five variations (L26R, P52L, A106D, A237E, and F440L) are described here for the first time (Table II).

The new variants present the following characteristics: L26R, which affects the second nucleotide of codon 26, substitutes leucine, an aliphatic and hydrophobic residue, with a polar and hydrophilic arginine. The variation L26R is present in patient 16 with the known mutation R368H. A237E presents a substitution of an alanine, weakly hydrophobic with small nonpolar side chains, with glutamic acid that carries a hydrophilic acidic group with strong negative charge; it was found in patient 179 associated with the insertion 1581-1582ins10.

A106D was observed on three PCG chromosomes (patient 110 is homozygous and patient 164 is compound heterozygote with the known mutation 1775-1801dup27). This change
causes a missense substitution of alanine (weakly hydrophobic with small nonpolar side
chains) with asparagine (with polar, uncharged side chains).

P52L changes proline, often present in the protein turning point and with small nonpolar side
chains, to leucine (aliphatic and hydrophobic). F440L changes phenylalanine, a hydrophobic
aminoacid usually orientated towards the interior of the folded protein, to leucine with the
same characteristics as phenylalanine.

The variations P52L and F440L are present in cis in patients 31 and 206, who are also
compound heterozygotes for other published pathological mutations (G61E and R390S
respectively); these patients are from the same Italian region.

All new identified aminoacidic changes alter residues that are evolutionarily conserved in
Eukariota and in the cytochrome P450 family (Fig 1 and 2), except Alanine in position 237 that
is conserved in Eukariota but not in the P450 family.

The most frequent mutations in our PCG patients are G61E, 1775-1801dup27, 1436-
1448del13 and 1236-1237insC; they occur respectively in 20.5%, 13.6%, 11.4% and 11.4% of
mutated chromosomes (Table II).

Two nucleotide variants were found in the untranslated exon 1: one (cDNA 198 C/T) was
characterised as a single nucleotide polymorphism (NCBI SNPs databank rs 9341244), the
other (C>A at 226 of cDNA) was identified for the first time in patient 91.

In these 72 Italian PCG subjects we also identified 6 Single Nucleotide Polymorphisms
(SNPs) previously described (10, 15, 23) and 2 new synonymous changes (G/A 359E and
C/T 363V) whose frequencies are 1/144 and 3/144 respectively.

**CYP1B1 SNP Haplotypes**

We analysed the following known CYP1B1 polymorphisms (10, 15, 25) in our patients:
g.3947C>G  (R48G),  g.4160G>T  (A119S),  g.8131C>G  (L432V),  g.8184C>T  (449D),
The most frequent haplotype in individuals with CYP1B1 mutations is 5'-C-G-G-T-A-3', as already described in other populations (6, 21, 26, 27). This haplotype is present in 41 of the 44 mutated alleles (93%), and in only 20 of the remaining 100 wild type alleles (20%); the difference is statistically significant (chi square = 22.23, p < 0.0001, CI95% =0.555-0.905).

Considering the seventeen mutations we found, fifteen are linked to the 5'-C-G-G-T-A-3' haplotype and two (E387K and R390S) are associated with two other different haplotypes.

**MYOC/TIGR gene analysis**

The report in published papers of patients with mutations in either CYP1B1 and MYOC genes induced us to look for MYOC/TIGR variations in the six patients having only one mutation in CYP1B1.

The analysis of the complete MYOC gene transcript identified, in three patients, two transitions (G>A at 249 and C>T at 1362 cDNA positions) causing the two aminoacidic variations R76K and A447V. Lysine in position 76 is similar to Arginine; Valine in position 447, an aminoacid with a large aliphatic hydrophobic side chain, usually located inside the protein, substitutes Alanine which has only small steric limits and can be located in the hydrophilic areas either outside or in the hydrophobic areas inside the protein.

In patient 42 the A447V is present with the G61E common CYP1B1 mutation; the two mutations are distinctly inherited from the two parents. Patient 132, with a single maternally transmitted CYP1B1 G61E mutation, inherited R76K from the father. Patient 203 received both the R76K and the CYP1B1 A443G from the unaffected father, who is homozygote for R76K.

To confirm the polymorphic role of R76K, already suggested in literature (24), we analysed 100 chromosomes in a random Italian population, obtaining a frequency of 0.125 and 0.875
for the A and G alleles respectively.
Discussion

Molecular analysis of the CYP1B1 transcript gene performed on Italian PCG patients found eighteen different variations in 34.7% of our pathological population, a percentage similar to which found in other countries. Also in Italian population the most frequent mutation is G61E. The new variations here identified (L26R, P52L, A106D, A237E) produce non synonymous aminoacidic changes and their pathological role in PCG development could be an explanation for the significant changes in the protein caused by the aminoacidic substitutions in Eukariota and cytochrome P450 family conserved regions. These changes are in a compound heterozygous state with already described pathological mutations and are distributed in exon numbers 2 and 3. Another new conservative aminoacid change, F440L, always found in cis with the P52L in 4 subjects, both in patients (with another CYP1B1 mutation) and in healthy carriers, could be classified as a rare polymorphism linked to the pathological P52L.

One of the two nucleotide variants we identified in the untranslated exon 1 had not been previously reported. In patient 91 a C to A at position 226cDNA was found combined with the pathological mutation 1236-1237insC. The presence in cis with the PCG mutation also in the unaffected mother suggests that this ex1UTR variation does not play a pathological role in PCG development.

We identified two new synonymous changes (G >A at the Glutamic Acid in position 359 and C>T at the Valine in position 363) in 2 different patients. These variations do not introduce aminoacidic changes and were not found in 100 analysed chromosomes, so we can consider them as rare polymorphisms.
Clinical evaluation of patients cannot show a particular phenotype-genotype correlation even if mutations that produce truncated proteins seem more frequent in patients with a disease onset before the first year of life and with more than 2 surgery events.

As described also in Indian and Saudi Arabian populations, we identified in three of our PCG patients the M1T and W57X CYP1B1 mutations, previously associated with Peters’ anomaly only. These results support the idea that distinct CYP1B1 mutations are not responsible for the two different diseases and that a specific phenotype could result from interactions between CYP1B1 and other modifier genes.

The previously described CYP1B1 intragenic polymorphisms (R48G, A119S, L432V, D449D and N453S) were analysed: the most frequent haplotype (93%) among affected individuals with CYP1B1 mutations appears to be 5'-C-G-G-T-A-3', which has been associated with fifteen different mutations. This particular haplotype was also observed in 94.7% of the Saudi Arabian and in the majority of the Brazilian PCG chromosomes. Interestingly, in our 47 PCG patients without mutations in CYP1B1, the frequency of the 5'-C-G-G-T-A-3' haplotype is the same as in the general Italian population (23% vs. 20%). The association of this specific uncommon haplotype with the majority of CYP1B1 mutations in geographically and ethnically distinct populations could confirm the hypothesis that this DNA sequence predisposes the gene to mutational events, as already suggested.

The presence, in other populations, of patients with mutations in both CYP1B1 and MYOC genes induced us to analyse the six patients with one mutation only in CYP1B1 for the presence of MYOC/TIGR variations. We found the R76K variation in compound heterozygosis in patients 132 and 203, who have the G61E and A443G CYP1B1 mutations respectively. The association of one CYP1B1 mutation with the MYOC R76K variation both alleles in the unaffected father of patient 203 defines the non-pathological role of this variation. It can be
confirmed as a polymorphism in the Italian population, with a frequency of 0.125 and 0.875 for the A and G alleles respectively.

In patient 42 we found, in double heterozygous status with the CYP1B1 G61E, the new MYOC variation A447V. This substitution affects a conserved aminoacidic position and introduces a residue with peculiar characteristics that can alter the protein functions. In this patient, the two mutations have been distinctly inherited from the unaffected heterozygous parents. A447V frequency in general population appears to be low, since it was not found in 100 control chromosomes. This is the first report of CYP1B1 and MYOC mutations in Italian patients affected by primary congenital glaucoma.

The molecular characterisation of both CYP1B1 and MYOC/TIGR genes in affected subjects allowed us to better clarify the genetic basis of the PCG disease. Our results confirm the major role of CYP1B1 in congenital glaucoma and propose also an autosomic recessive role of MYOC/TIGR gene in a digenic inheritance model. However, the presence of only one CYP1B1 defect in 5 patients confirms that other genes could interact with the function of the CYP1B1 codified protein in PCG onset with different digenic inheritances.
Table 1: Phenotype–Genotype correlation in Italian PCG patients with CYP1B1 mutations

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<th>N° of Surgery events</th>
<th>Genotype</th>
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<th>SNP 2</th>
<th>SNP 3</th>
<th>SNP 4</th>
<th>SNP 5</th>
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<td>4</td>
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<td>G</td>
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<td>A</td>
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<td>3</td>
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<td>G</td>
<td>G</td>
<td>T</td>
<td>A</td>
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<td>T</td>
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<td>[1436-1448del13] / -</td>
<td>C</td>
<td>G</td>
<td>G</td>
<td>T</td>
<td>A</td>
</tr>
<tr>
<td>132</td>
<td>3 y</td>
<td>0</td>
<td>[G61E] / -</td>
<td>C</td>
<td>G</td>
<td>G/C</td>
<td>T/C</td>
<td>A/G</td>
</tr>
</tbody>
</table>

SNP 1-5: 1. 3947 C>G (R48G); 2. 4160 G>T (A119S); 3. 8131C>G (L432V); 4. 8184C>T (D449D); 5. 8195A>G (N435S).
### Table II: Frequencies of CYP1B1 mutations in Italian PCG patients

<table>
<thead>
<tr>
<th>Exon</th>
<th>Mutation</th>
<th>Effect</th>
<th>Patients</th>
<th>N (%) of affected chromosomes (n=44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>T374C</td>
<td>M1T</td>
<td>54</td>
<td>1 (2.3%)</td>
</tr>
<tr>
<td>2</td>
<td>T449G</td>
<td>L26R</td>
<td>16</td>
<td>1 (2.3%)</td>
</tr>
<tr>
<td>2</td>
<td>C527T</td>
<td>P521*^</td>
<td>31, 206</td>
<td>2 (4.5%)</td>
</tr>
<tr>
<td>2</td>
<td>G543A</td>
<td>W57X</td>
<td>1, 2</td>
<td>2 (4.5%)</td>
</tr>
<tr>
<td>2</td>
<td>G554A</td>
<td>G61E</td>
<td>12, 31, 67, 89, 122, 153, 42, 131</td>
<td>9 (20.5%)</td>
</tr>
<tr>
<td>2</td>
<td>C689A</td>
<td>A106D^</td>
<td>110, 164</td>
<td>3 (6.8%)</td>
</tr>
<tr>
<td>2</td>
<td>C1082A</td>
<td>A237E^</td>
<td>179</td>
<td>1 (2.3%)</td>
</tr>
<tr>
<td>2</td>
<td>1236-1237insC</td>
<td>Frameshift beginning from aa 288</td>
<td>12, 71, 89, 91, 161</td>
<td>5 (11.4%)</td>
</tr>
<tr>
<td>3</td>
<td>C1435T</td>
<td>R355X</td>
<td>62, 63</td>
<td>2 (4.5%)</td>
</tr>
<tr>
<td>3</td>
<td>1436-1448del13</td>
<td>Frameshift beginning from aa 355</td>
<td>67, 113, 114, 153, 161</td>
<td>5 (11.4%)</td>
</tr>
<tr>
<td>3</td>
<td>G1475A</td>
<td>R368H</td>
<td>16, 31</td>
<td>2 (4.5%)</td>
</tr>
<tr>
<td>3</td>
<td>G1531A</td>
<td>E387K</td>
<td>62, 63</td>
<td>2 (4.5%)</td>
</tr>
<tr>
<td>3</td>
<td>C1540A</td>
<td>R390S</td>
<td>206</td>
<td>1 (2.3%)</td>
</tr>
<tr>
<td>3</td>
<td>1581-1582ins10</td>
<td>Frameshift beginning from aa 404</td>
<td>179</td>
<td>1 (2.3%)</td>
</tr>
<tr>
<td>3</td>
<td>T1692G</td>
<td>F440L*^</td>
<td>31, 206</td>
<td>1 (2.3%)</td>
</tr>
<tr>
<td>3</td>
<td>C1700G</td>
<td>A443G</td>
<td>203</td>
<td>1 (2.3%)</td>
</tr>
<tr>
<td>3</td>
<td>1775-1801dup27</td>
<td>Frameshift beginning from aa 468</td>
<td>1, 2, 25, 71, 164</td>
<td>6 (13.6%)</td>
</tr>
</tbody>
</table>

* Present on the same chromosome inherited from the unaffected father, in patients 31 and 206 who are also compound heterozygotes for other published pathological mutations (G61E and R390S respectively).

^ New variations
Fig. 1 Aminoacidic Sequence Alignment of CYP1B1 and homologous genes in Euakariota.

Lane 1: CYP1B1 H. Sapiens, lane 2: LOC483038 C. Familiaris, lane 3: Cyp1b1 M. Musculus, lane 4: LOC421466 G. Gallus, lane 5: spo D. Melanogaster
Fig. 2 Aminoacidic Sequence Alignment of CYP1B1 (second lane) and other P450 family members (CYPb2b4, CYP17A1, CYP22C22, CYP2C3, CYP2C7, CYP2C1, CYP2C23, CYP2D14, CYP2D4).
REFERENCES


15) Stoilov, I.; Akarsu, A. N.; Alozie, I et al. Sequence analysis and homology modeling suggest that primary congenital glaucoma on 2p21 results from mutations disrupting either the hinge region or the conserved core structures of cytochrome P4501B1. *Am J Hum Genet.* 1998;62:573-584.


19) Chakrabarti S, Kaur K, Komatireddy S et al. Gln48His is the prevalent myocilin mutation in primary open angle and


