Identification of Novel Mutations Causing Familial Primary Congenital Glaucoma in Indian Pedigrees

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PURPOSE. To determine the possible molecular genetic defect underlying primary congenital glaucoma (PCG) in India and to identify the pathogenic mutations causing this childhood blindness.

METHODS. Twenty-two members of five clinically well-characterized consanguineous families were studied. The primary candidate gene CYP1B1 was amplified from genomic DNA, sequenced, and analyzed in control subjects and patients to identify the disease-causing mutations.

RESULTS. Five distinct mutations were identified in the coding region of CYP1B1 in eight patients of five PCG-affected families, of which three mutations are novel. These include a novel homozygous frameshift, compound heterozygous missense, and other known mutations. One family showed pseudodominance, whereas others were autosomal recessive with full penetrance. In contrast to all known CYP1B1 mutations, the newly identified frameshift is of special significance, because all functional motifs are missing. This, therefore, represents a rare example of a natural functional CYP1B1 knockout, resulting in a null allele (both patients are blind).

CONCLUSIONS. The molecular mechanism leading to the development of PCG is unknown. Because CYP1B1 knockout mice did not show a glaucoma phenotype, the functional knockout identified in this study has important implications in elucidating the pathogenesis of PCG. Further understanding of how this molecular defect leads to PCG could influence the development of specific therapies. This is the first study to describe the molecular basis of PCG from the Indian subcontinent and has profound and multiple clinical implications in diagnosis, genetic counseling, genotype-phenotype correlations and prognosis. Hence, it is a step forward in preventing this devastating childhood blindness. (Invest Ophthalmol Vis Sci. 2002;43:1358–1366)
### TABLE 1. Clinical Data of Subjects with Primary Congenital Glaucoma

<table>
<thead>
<tr>
<th>Pedigree</th>
<th>Age of Onset</th>
<th>Age of Diagnosis</th>
<th>Presence of Haab's Striae</th>
<th>Corneal Diameter (mm) and Clarity at Diagnosis (OD; OS)</th>
<th>IOP at Diagnosis (mm Hg OD; OS)</th>
<th>Last C/D Ratio (OD; OS)</th>
<th>Last Visual Acuity (OD; OS)</th>
<th>Treatments (OD; OS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCG 4</td>
<td>By birth</td>
<td>2 wk</td>
<td>Present in OU</td>
<td>12; 12.5 Buphthalmos OU; hazy cornea and edema</td>
<td>36; 38</td>
<td>0.9; NA</td>
<td>NPL OU</td>
<td>Medical and 1× Trab/Trab OU; 1× PK* OD</td>
</tr>
<tr>
<td></td>
<td>Affected sibling</td>
<td>By birth</td>
<td>3 mo</td>
<td>NA OU</td>
<td>NA; Buphthalmos OU; hazy cornea and atrophic</td>
<td>NA OU</td>
<td>NA OU</td>
<td>NPL OU</td>
</tr>
<tr>
<td>PCG 11, Proband</td>
<td>By birth</td>
<td>2 wk</td>
<td>Absent OU</td>
<td>12; 12.5 Corneal edema OU</td>
<td>30 OU</td>
<td>NA OU</td>
<td>Fixing and following light OU</td>
<td>Medical and 1× Trab/Trab OU; 2× Trab/Trab OS</td>
</tr>
<tr>
<td>PCG 1</td>
<td>Proband</td>
<td>By birth</td>
<td>Absent OU</td>
<td>24 OU</td>
<td>0.8; 0.9</td>
<td>20/25 OU</td>
<td>Medical treatment OU</td>
<td>Medical treatment OD</td>
</tr>
<tr>
<td></td>
<td>Affected mother</td>
<td>Late onset in OD; &gt;3 years</td>
<td>Absent OD; present OS</td>
<td>NA; clear OU</td>
<td>NA; Clear OD; hazy OS</td>
<td>34; 50</td>
<td>0.8; 0.9</td>
<td>20/20; NPL</td>
</tr>
<tr>
<td>PCG 2, Proband</td>
<td>By birth</td>
<td>2 wk</td>
<td>Present OU</td>
<td>13 OU Buphthalmos OU; hazy cornea OU</td>
<td>NA OU</td>
<td>0.9 OU</td>
<td>20/30; PL</td>
<td>3× Trab/Trab OU; retinal reattachment surgery OS†; medical treatment OD</td>
</tr>
<tr>
<td>PCG 6</td>
<td>Proband</td>
<td>By birth</td>
<td>Absent OU</td>
<td>13; 12.5 Corneal edema OU</td>
<td>26; 30</td>
<td>0.3 OU</td>
<td>20/40; 20/200</td>
<td>1× Trab/Trab OU</td>
</tr>
<tr>
<td></td>
<td>Affected sibling</td>
<td>By birth</td>
<td>3 mo</td>
<td>Absent OU</td>
<td>15 OU Corneal edema and scarring OU</td>
<td>32 OU</td>
<td>NA OU</td>
<td>PL; HM</td>
</tr>
</tbody>
</table>

IOP, intraocular pressure; OD, right eye; OS, left eye; OU, both eyes; C/D, cup-disc ratio of the optic nerve; NPL, no perception of light; PL, perception of light; HM, hand motion; NA, not available; X, Times; Trab/Trab, combined trabeculotomy and trabeculectomy; PK*, penetrating keratoplasty performed but resulted in graft failure; OS† left eye became atrophic.
2). Amplicons were sequenced directly, and the patient and control sequences were compared to identify all mutations. The primers used were as follows: set I (1 forward [F]/1 reverse [R], 786 bp), set II (2F/2R, 648 bp), set III (3F/3R, 885 bp). All PCRs were performed for only 30 cycles, and conditions for sets I and II were as reported earlier.1,2; conditions for set III are given in Table 2. Twenty-five to 50-μL polymerase chain reactions (PCR) were performed with the following: 50 to 100 ng genomic DNA, 1× PCR buffer with 1.5 to 2.0 mM MgCl₂, 200 μM dNTPs, 0.5 μM of each primer, and 1 U Taq polymerase (Bangalore Genei, Bangalore, India), with or without 10% dimethyl sulfoxide (DMSO). Primer sets I and II had 10% DMSO and 1.5 mM MgCl₂, whereas set III had only 2.0 mM MgCl₂. The same sets of primers were used for PCR and bidirectional sequencing. The three amplicons were purified (pre-PCR sequencing kit; USB, Cleveland, OH), terminator cycle sequencing was performed (BigDye kit; PE-Applied Biosystems, Foster City, CA), and sequencing reactions were performed on an automated DNA sequencer (ABI model 377; PE-Applied Biosystems).

**PCR-Restriction Fragment Length Polymorphism Analyses and Cosegregation of Mutant Alleles with Disease Phenotype**

In all cases, mutations resulted in either loss or gain of recognition sites (Table 2). For determining the cosegregation of mutant alleles with disease phenotype in the family, the respective fragment harboring the mutation was amplified from all family members, and an aliquot of amplicons was digested with the corresponding restriction enzymes (Table 2; MBI Fermentas, Vilnius, Lithuania). The fragments were separated on 8% polyacrylamide gel, stained with ethidium bromide and visualized to distinguish the wild type and mutant alleles. Seventy volunteer donors without history of eye disorders served as control subjects.

**RESULTS**

**Identification of Pathogenic Mutations**

**Novel Frameshift Mutation and Functional Null Allele.** In family PCG4 (an uncle-to-niece marriage) two patients showed a homozygous insertion (Figs. 1A, 1B; Table 2) of a nucleotide A at cDNA position 376 (376insA). This novel mutation, not previously reported, resulted in a frameshift that truncated the open reading frame (ORF) by creating a premature stop codon (TGA), 636 bp downstream from this insertion. Consequently, a truncated 222-amino-acid (aa) protein missing 321 aa from the C terminus was generated (Fig. 1C). This also abolished the restriction site Eco130I in exon II. Both the wild-type and the mutant proteins contained just 10 aa at the N terminus, which is similar in both, and the frameshift eliminated all CYP1B1 domains, resulting in a functional null allele. All unaffected members in family PCG4 were heterozygous for this mutation (Fig. 2A).

**Novel Compound Heterozygous Mutations and Pseudodominance.** In another family (PCG1; marriage between first cousins), parent-to-child transmission of the disease was noticed. This is an interesting pedigree in which the daughter (proband) and mother were affected with bilateral PCG and the father was a normal carrier (Fig. 3A). Two affected generations showed varying severity and manifestations. The mother showed asymmetric manifestation (left eye blind, right eye mildly affected), whereas the proband displayed a uniform milder manifestation in both eyes. The proband had a novel compound heterozygous missense mutation (Table 2) within exon II. The first mutation (Fig. 3B) was a C→T substitution at 923 bp, resulting in a proline-to-leucine change at aa 193 (P193L) and a gain of the restriction site Eco81I. The second

<table>
<thead>
<tr>
<th>Pedigree</th>
<th>Mutation Type</th>
<th>Mutation Site Change</th>
<th>Codon Change</th>
<th>Restriction Site Change</th>
<th>Diagnostic Method</th>
<th>Primers Used for PCR and Sequencing</th>
<th>Novel or Reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCG2</td>
<td>Novel†</td>
<td>1360 Panicker et al.</td>
<td>I 923G</td>
<td>Heterozygous</td>
<td>–</td>
<td>1F-tctccagagagtcagctccg (3676–3695)</td>
<td>Novel*</td>
</tr>
<tr>
<td>PCG3</td>
<td>Novel†</td>
<td>1360 Panicker et al.</td>
<td>II 959G</td>
<td>Heterozygous</td>
<td>–</td>
<td>2F-tcccagaaatattaatttagtcaactg (7740–8605)</td>
<td>Novel*</td>
</tr>
<tr>
<td>PCG4</td>
<td>Novel*</td>
<td>1360 Panicker et al.</td>
<td>II 985G→A</td>
<td>Heterozygous</td>
<td>–</td>
<td>3F-tcccagaaatattaatttagtcaactg (7740–8605)</td>
<td>Novel*</td>
</tr>
<tr>
<td>PCG5</td>
<td>Novel*</td>
<td>1360 Panicker et al.</td>
<td>II 1125C→T</td>
<td>Heterozygous</td>
<td>–</td>
<td>4F-tctccagagagtcagctccg (3676–3695)</td>
<td>Novel*</td>
</tr>
<tr>
<td>PCG6</td>
<td>Novel*</td>
<td>1360 Panicker et al.</td>
<td>II 1140G→A</td>
<td>Heterozygous</td>
<td>–</td>
<td>5F-tctccagagagtcagctccg (3676–3695)</td>
<td>Novel*</td>
</tr>
<tr>
<td>PCG7</td>
<td>Novel*</td>
<td>1360 Panicker et al.</td>
<td>II 1140G→A</td>
<td>Heterozygous</td>
<td>–</td>
<td>6F-tctccagagagtcagctccg (3676–3695)</td>
<td>Novel*</td>
</tr>
<tr>
<td>PCG8</td>
<td>Novel*</td>
<td>1360 Panicker et al.</td>
<td>II 1140G→A</td>
<td>Heterozygous</td>
<td>–</td>
<td>7F-tctccagagagtcagctccg (3676–3695)</td>
<td>Novel*</td>
</tr>
<tr>
<td>PCG9</td>
<td>Novel*</td>
<td>1360 Panicker et al.</td>
<td>II 1140G→A</td>
<td>Heterozygous</td>
<td>–</td>
<td>8F-tctccagagagtcagctccg (3676–3695)</td>
<td>Novel*</td>
</tr>
<tr>
<td>PCG10</td>
<td>Novel*</td>
<td>1360 Panicker et al.</td>
<td>II 1140G→A</td>
<td>Heterozygous</td>
<td>–</td>
<td>9F-tctccagagagtcagctccg (3676–3695)</td>
<td>Novel*</td>
</tr>
</tbody>
</table>
Homozygous Missense Mutations. Three families were identified with two known homozygous missense mutations\(^{15,23}\); two with the R368H homozygous mutation and one with the G61E homozygous mutation (Table 2). Both are highly conserved across various members of the cytochrome P450 superfamily (Fig. 4). These mutations were found to segregate with four patients (families PCG2 and PCG11, one patient each; PCG6, two patients) in three unrelated consanguineous families (PCG2 and PCG6, first-cousin marriage; PCG11, uncle-to-niece marriage). Consistent with recessive inheritance, mutant alleles segregated with disease phenotypes in all families.

Patients in families PCG2 and PCG6 showed the same homozygous mutation: G→A substitution at 1449 bp. This resulted in an arginine-to-histidine change at aa 368 (R368H) in CYP1B1 and a loss of restriction site TaqI in exon III (Table 2). In PCG11, substitution of a nucleotide G→T at cDNA position 528 resulted in a glycine-to-glutamic acid replacement at aa 61 (G61E) of CYP1B1 and a gain of the restriction site TaqI in exon II (Table 2).

Nonpathogenic CYP1B1 Single Nucleotide Polymorphisms

In addition to pathogenic mutations five other single nucleotide polymorphisms (SNPs; Table 3) were identified in the less conserved region of CYP1B1. Because PCG6 had two different homozygous missense mutations, the highly conserved residue (R368H, reported earlier\(^{15}\)) was considered to be a pathogenic mutation, whereas the less conserved one (G184S) was taken to be a novel polymorphism (Fig. 4).

**Structural Implications of Mutant Proteins**

It is interesting to note that, of the four amino acid mutations (excluding insertion mutation), three occur in the less-conserved N-terminal domain of the protein. An alignment of the amino acid sequence with a homologue of known three-dimensional structure (Protein Data Bank [PDB] code: 1DT6) revealed that all the mutation sites are away from the heme-binding pocket and therefore probably do not affect directly the binding of the heme. However, these sites seem to be important in maintaining the structural integrity of the protein. The conserved glycine residue at position aa 61 is in a left-hand helical conformation and is in a very unique position where the peptide chain takes a sharp turn. Position aa 193 forms the N-capping region of the helix (aa 173–210) and is most suited for proline, which is also highly conserved. Any amino acid change at this position may disrupt the helical structure. The same is probably true for the position E229, which is in the middle of the helix (aa 218–234). R368 is probably less important structurally, because the site is in the loop region, which is on the surface of the protein and is probably necessary for protein–protein interactions.

Our examination of the translated product of the frameshift mutation (576insA) revealed that the amino acid sequence of the mutant allele (P193L) maps to a region highly conserved among various types of cytochromes, whereas the E229K mutation is conserved only among the CYP1B1 types (Fig. 4). Screening of 70 control subjects by PCR-restriction fragment length polymorphism (RFLP) not only confirmed the absence of this compound heterozygous mutation in the normal population, but also supports that it is likely to be pathogenic. However, a few control subjects (12.8%) were heterozygous for the 923C→T (E229K) mutation, but none for the 959G→A (P193L) mutation.

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**Figure 2.** PCR-RFLP analyses of the cosegregation of different mutations with disease phenotypes. Filled squares and circles: Affected individuals; arrow: probands; dot in open symbol: carriers; double line: consanguinity. DNA molecular weight marker (lane M) in base pairs (left); allele sizes (right); control (lane C); mutant allele(s) (arrow/arrowheads). Restriction site changes and mutations (nucleotide as well as amino acid changes) are shown at the bottom of each panel. (F) Sample for analysis unavailable. (A) Wild-type allele amplification and restriction digestion of amplicon from control DNA generated 384- and 158-bp fragments (lane C). Mutation abolishes the EcoRI site. In heterozygous individuals (carriers) in addition to the wild-type allele, a mutant allele of 542 bp was present. In the disease phenotype (homozygous) only a mutant allele of 542 bp was evident. (B) C→T substitution in PCG1 results in a gain of an EcoRI site, which is evident from the cleavage of the 648-bp fragment (lane C) into 523-bp and 125-bp fragments. In carriers, in addition to the wild-type allele, a mutant allele of 648 bp was present. (C) Restriction digestion of the wild-type allele in the control generated 359- and 229-bp (lane C) fragments and abolished the Eam1104I site. In carriers, in addition to the wild-type allele 588, mutant alleles of 359 and 229 bp were present. (D) Restriction digestion of the wild-type allele in the control showing undigested fragment of 627 bp (lane C). Mutation creates a TaqI site. In carriers, in addition to the wild-type allele, mutant alleles of 318 and 309 bp were present. In the disease phenotype (homozygous) only mutant alleles of 318 and 309 bp were present. (E, F) Restriction digestion of wild-type allele in the control generated 507- and 200-bp fragments (lane C). Mutation creates a TaqI site. In carriers, in addition to the wild-type allele, a mutant allele of 352 bp was present. In the disease phenotype (homozygous) only mutant alleles of 507 and 352 bp were present.

The new ORF does not show an appreciable match with any of the known protein sequences in the PDB. A secondary structure prediction of the sequence showed that the translated product is mostly made of coiled regions.

**Genotype–Phenotype Correlations**

Correlation between genotype and phenotype based on this study was evident from a comparison of the different mutations associated with varying manifestations and phenotypes of the disease (Table 4). The PCG phenotypes associated with various mutations showed varying severity and manifestations. In some cases, there was asymmetric manifestation between eyes of the patients (mother in family PCG1), whereas the same mutation (R368H) exhibited interfamily (families PCG2 and -6) as well as intrafamily (family PCG6) variability (Tables 1, 4).

**DISCUSSION**

This is the first genetic study from India to describe the molecular defect underlying the PCG phenotype and demonstrates the direct association of the CYP1B1 mutations with this devastating childhood blindness.22 Unknown developmental defects of the trabecular meshwork and anterior chamber angle of the eye cause this disorder.10,21,24 In our investigation of five consanguineous PCG-affected Indian families, five pathogenic mutations (including three novel ones) were identified in eight affected members. These include a novel homoygous frameshift mutation resulting in a functional null allele and compound heterozygous missense and known missense mutations (Table 2). That all are disease-causing mutations is shown by the fact that all mutant alleles cosegregate with the disease phenotype and are absent in the normal population and that the mutated residues are highly conserved across various members of the cytochrome P450 superfamily (Fig. 4). In addition, five SNPs were found in the affected families. These were either observed in the general population and/or were found to affect poorly conserved amino acid residues exclusively (Fig. 4). This study also indicates that CYP1B1 could be the predominant cause of PCG in the Indian ethnic background, because all families analyzed so far have had mutations in this gene.

Pseudodominant inheritance was seen in one family, whereas all others showed autosomal recessive inheritance with full penetrance. All patients inherited two mutant alleles, whereas unaffected members were heterozygous (carriers) for a single mutant allele segregating in that particular family, except in the pseudodominant family (Fig. 2).
Of all mutations identified herein, the frameshift mutation resulted in the most severe phenotype. Only the first 10 aa of the 543-aa CYP1B1 protein remain unchanged by the frame-shift, whereas the remainder of the protein was replaced by an out-of-frame polypeptide of 222 aa. Despite maximum medical and prompt surgical treatments, both patients in family PCG4 exhibited a most devastating phenotype and were blind (Fig. 5).

In all PCG-pseudodominant families reported so far, the affected parent has been homozygous and the other a normal carrier; but analysis of the present pseudodominant family (PCG1) indicates that the affected parent (II.1) is a compound heterozygote. Moreover, an interesting observation is that probably there are three compound heterozygous individuals (II.1, III.1, and III.2) in this family, all segregating with different combinations of mutant alleles (Fig. 3) with varying expression, of which one exhibits normal phenotype (unaffected sibling [III.2]—a glaucoma suspect). The exact age of onset of the disease in this case was difficult to ascertain because the affected status of the mother (II.1) was revealed through her daughter (the proband [III.1]). The presence of Haab’s striae in the left eye of the affected mother (Table 1) suggests that she had PCG in that eye before 3 years of age, whereas the right eye had late-onset PCG. An asymmetric manifestation of PCG was seen in the affected mother (the left eye became blind at 21 years, whereas IOP in the right eye is under control with medication).

The mother had glaucoma diagnosed at age 30 (Table 1) and had ocular features indicating that disease may have begun in one eye before age 3. However, because the second CYP1B1 mutation has not been identified in the mother (II.1), and this missing allele, as passed on to her 8-year-old daughter (III.2), has resulted in a normal phenotype (Fig. 3A), this seems to be a complex situation, for which various plausible explanations...
can be considered: (1) The dramatic phenotypic variability observed between the two eyes of the affected mother is possibly the consequence of an as yet unknown mutation within the promoter region (perhaps a promoter deletion), and may indicate that CYP1B1 is a dosage-sensitive gene. (2) The mother may simply be a carrier of congenital glaucoma who happens also to have an early-onset form of glaucoma caused by mutation at another locus or glaucoma of a nongenetic origin. (3) It may be possible that heterozygosity for the 925C→T mutation causes late-onset disease, although to our knowledge there are no reported instances of development of late-onset disease in carriers of the CYP1B1 mutation. (4) If the mother has a new mutation and is mosaic for the mutation, she could have one eye more affected than the other, because of unequal representation of the defect in the two eyes. It is possible that she has an unaffected child who inherited that chromosome, because of the absence of the mutation in the germ line. Although various roles for CYP1B1 in eye development have been proposed recently,\textsuperscript{23} it is tempting to speculate that the likely role of CYP1B1 is in the detoxification or elimination of a toxic metabolite, which may be harmful to the normal development of the eye.

Previous studies have indicated that the G61E and R368H mutations are not fully penetrant in Saudi families,\textsuperscript{10,15} whereas in these Indian families, both are fully penetrant. R368H, reported earlier,\textsuperscript{15} maps to helix K, which is one of the highly conserved core structures (CCSs). This homozygous mutation seen in three patients of two unrelated families (PCG2 and PCG6) shows a very severe phenotype, in either one or both eyes. The CCSs are suspected to be involved in proper protein folding and in active heme binding.\textsuperscript{23} Therefore, any homozgyous impairment of this domain could lead to a severe phenotype. The other highly conserved G61E mutation\textsuperscript{12} is adjacent to the N-terminal proline-rich region of CYP1B1 and is also likely to affect the proper protein function and result in disease manifestation. The proline-proline-glycine-proline motif may serve to join the membrane-binding N terminus to the globular region of the P450 protein.\textsuperscript{9,10,15,23}

Because the anterior chamber angle in humans has undergone some very recent evolutionary changes, this may be a problem in using animal models, especially the CYP1B1 knockout mice, for studying PGCLs pathogenesis.\textsuperscript{23} Typical trabecular meshwork can be found only in humans and higher primates, whereas lower species have only a reticular meshwork.\textsuperscript{24} Although it may be difficult to extrapolate the findings obtained from the CYP1B1 null mice, the phenotype obtained in such mice need not be the same as that of the functional CYP1B1 knockout identified in the present study. This view is in fact substantiated by a study, wherein it was demonstrated that CYP1B1 null mice did not show any obvious blindness or evidence of glaucoma, as assessed by standard behavioral comparisons with wild-type mice in their response to light and dark.\textsuperscript{25} Furthermore, a frequent observation in various knockout studies is that the phenotypes do not transfer identically across species.

The information derived from this study has both basic and clinical relevance. Genetic counseling can be provided to at-risk families that will aid in the prevention of PGCL-related blindness. The characterization of CYP1B1 and the spectrum of mutations with evidence of pathogenicity and high pen-

\begin{table}[h]
\centering
\caption{Genotype/Phenotype Effect}
\begin{tabular}{|l|l|l|l|l|}
\hline
Pedigree & Mutation & Laterality & Severity* (OD; OS) & Prognosis (OD; OS) \\
\hline
PCG4 & & & & \\
Proband & Ter@223 aa & Bilateral & OU very severe† & OU very poor \\
Affected sibling & Ter@223 aa & Bilateral & OU very severe† & OU very poor \\
\hline
PCG11, proband & G61E & Bilateral & OU mild & OU good \\
\hline
PCG1 & & & & \\
Proband & P193L and E229K & Bilateral & OU mild & OU good \\
Affected mother & P193L† & Bilateral with late onset OD & OD normal & OD good \\
\hline
PCG2 & & & & \\
Proband & R368H & Bilateral & OS very severe† & OS very poor \\
Affected sibling & R368H & Bilateral & OD mild & OD good \\
\hline
PCG6 & & & & \\
Proband & R368H & Bilateral & OS very severe & OS very poor \\
\hline
\end{tabular}
\footnotetext{*}Severity of the disease is arbitrarily graded based on the corneal changes, IOP, cup-to-disc ratio, and last recorded visual acuity (20/20 is normal; <20/40–20/40 is mild; <20/40–20/200 is severe; <20/200–PL is very severe; NPL is blind; see Table 1.

†Affected individual is blind.

‡Second mutation in PCG1 mother is unknown.
\end{table}
etrance could have profound clinical implications in the management of PCG. This will facilitate prenatal diagnosis for this condition, which carries high life-long morbidity. Indeed, further screening of probands using the simple, fast, and inexpensive PCR-RFLP diagnostic methods developed in this study has enabled us to rapidly identify similar mutations in several other PCG-affected families (Reddy et al., manuscript in preparation). However, further analysis of more families with PCG is needed to determine the clinical correlation with the severity of the disease, if any.

Acknowledgments

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References


