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Mutation spectrum of the *CYP1B1* gene in Indian primary congenital glaucoma patients

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Purpose: The human Cytochrome P450 gene *CYP1B1* has been implicated in primary congenital glaucoma worldwide. The aim of this study was to understand the role of *CYP1B1* mutations in causing primary congenital glaucoma in Indian populations.
Methods: The study included 64 new and unrelated cases of primary congenital glaucoma from different ethnic groups of India. Direct sequencing screened the coding and the promoter regions of *CYP1B1*.
Results: Sixteen pathogenic mutations were observed in 24 cases, of which 7 were novel. These included two frameshift mutations leading to deletions of 23 bp (g.3905del23bp) and 2 bp (g.7900-7901delCG) in exons II and III, respectively. Four novel missense mutations viz. A115P, M132R, Q144P, S239R were noted in exon II, and one in exon III (G466D), whose residue is a part of the "signature sequence" (NH_2-FXXGXXXCXG-COOH) and is present in all heme binding cytochromes. Overall, *CYP1B1* was involved in 37.50% (24/64) cases and homozygosity of the mutant allele was seen in 29.68% (19/64) and compound heterozygosity in 3.12% (2/64) of the cases, respectively. The frequency of *CYP1B1* mutations was comparatively lower than Saudi Arabian, Slovakian Gypsys, and Turkish populations, largely due to genetic heterogeneity and ethnic diversities in Indian populations. Genotype-phenotype correlation indicated variable prognosis that could be due to the type of mutation, leading to alteration of CYP1B1 protein.
Conclusions: This study provides a mutation spectrum of *CYP1B1* causing primary congenital glaucoma in Indian populations that has implications in devising molecular diagnostics for rapid screening.

Primary congenital glaucoma (PCG; OMIM 231300) is an inherited ocular congenital anomaly of the trabecular meshwork and anterior chamber angle that leads to the obstruction of aqueous outflow, increased intraocular pressure (IOP), and optic nerve damage resulting in childhood blindness. The disease manifests in the neonatal or early infantile period with symptoms of photophobia, epiphora, and signs of enlargement of the globe, edema, opacification of the cornea, breaks in the Descemet's membrane, and others [1]. The prevalence of PCG varies across ethnic communities and geographical boundaries, ranging from 1 in 10,000-20,000 in the western populations [2] to 1 in 2,500 and 1 in 1,250 in the Saudi Arabian [3] and Gypsy populations of Slovakia [2], respectively. In the Indian state of Andhra Pradesh, prevalence is estimated to be around 1:3,300 and accounts for 4.2% of all childhood blindness [4]. The mode of inheritance is largely autosomal recessive with variable penetrance but rare cases of pseudodominance are also seen in families with multiple consanguinity [1]. Three chromosomal loci have been linked to PCG: *GLC3A* (2p21), *GLC3B* (1p36), and *GLC3C* (14q24.3) [5-7]. *GLC3A*, harboring the human cytochrome P450 gene *CYP1B1* (OMIM 601771), has been characterized [3]. More than 40 different mutations have been identified in *CYP1B1* to be causal for PCG in different ethnic backgrounds and populations highlighting the allelic heterogeneity of the condition [8,9].

The involvement of *CYP1B1* in other PCG populations varies from 20% in Indonesians and Japanese to 50% among the Brazilians and about 100% among the Saudi Arabians and Slovakian Gypsies [8,10-12]. Also it was shown that the Slovakian Gypsies and Saudi Arabian populations exhibited allelic homogeneity that was largely attributed to consanguinity and inbreeding [13,14]. Consanguinity and inbreeding are also prevalent in different ethnic groups of India [15] and thus provide an excellent opportunity to explore the role of *CYP1B1* causing PCG in these populations.

Earlier we reported the involvement of *CYP1B1* in 5 PCG families from India and devised PCR-based restriction fragment length polymorphism (RFLP) methods to detect these mutations [16]. With this strategy we further screened 138 PCG cases and found that 30.8% of these cases were positive for one of the previously identified six mutations and that R368H happened to be the most prevalent mutation in the Indian population [17]. In order to understand the spectrum of *CYP1B1*

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mutations causing PCG in Indian populations, we screened *CYP1B1* in 64 new and unrelated cases from different ethnic backgrounds.

METHODS

Clinical examination and selection of cases: The study protocols adhered to the guidelines of the Declaration of Helsinki. After approval of the Institutional Review Board, 64 consecutively diagnosed PCG cases from different ethnic backgrounds, presenting at the L. V. Prasad Eye Institute from January 2001 to June 2003 were recruited. All patients underwent slit lamp biomicroscopy, applanation tonometry, and gonioscopy (where corneal clarity permitted). General anesthesia (Savoflorane) was used in the younger age groups and where required. The inclusion criteria were increased corneal diameter (>12.0 mm) along with raised intraocular pressure (>21 mm Hg) and/or presence of Haab's striae, or optic disc changes (where examination was possible). Symptoms of epiphora, photophobia, and rupture in the Descemet's membrane were the corroborating factors. The age of onset ranged from 0-1 years. Clinical diagnosis was confirmed independently by two glaucoma surgeons (AKM and RT) with expertise in congenital glaucoma. Seventeen cases had a positive family history of the disease, while the rest were sporadic. Parental consanguinity was seen in 55% of these cases. One hundred ethnically matched normal individuals without any ocular disorders served as controls. Peripheral blood samples were collected from the probands, their relatives, and controls by venipuncture with prior informed consent.

Screening of CYP1B1 *gene:* DNA was extracted from the leukocytes following standard protocols [18]. The entire coding regions of *CYP1B1* were amplified using three sets of predesigned oligonucleotide primers with PCR conditions as described earlier [16]. The 485 bp upstream region (2,748 bp to 3,233 bp) flanking four important regions for maximum promoter activity was screened using a set of forward (5'-AGC GGC CGG GGC AGG TTG TAC C-3') and reverse (5'-ATT GGG ATG GGG ACG GAG AA-3') primers. PCR was performed with 25 µl reaction mixtures containing 50 ng of genomic DNA, 1X PCR buffer (containing 100 mM Tris-HCl, 500 mM KCl and 0.8% Nonidet P40) with $MgCl_2$, 200 mM of dNTPs, 0.5 µM of each primer, 10% DMSO, and 1 unit of Taq DNA polymerase (MBI Fermentas, Vilnius, Lithuania). The amplification conditions were an initial denaturation at 95 °C for 3 min followed by 30 cycles of denaturation, annealing and extensions at 95 °C (30 s), 62 °C (30 s), and 72 °C (45 s), respectively with a final extension at 72 °C for 7 min. The amplicons were purified using spin-columns (Sigma-Aldrich, St. Louis, MO) and subjected to bi-directional sequencing using BigDye terminator (version 3.1) chemistry according to the manufacturers protocol (Applied Biosystems, Foster city, CA). Sequencing was performed on an automated DNA sequencer (ABI 3100) and the data were analyzed with the SEQUENCING ANALYSIS software (Both from Applied Biosystems, Foster City, CA).

RESULTS

Identification of 7 novel mutations: Direct sequencing revealed 16 different mutations in 24 unrelated cases, of which 7 were novel. These included two frameshift mutations leading to deletions of 23 bp (g.3905del23bp, Figure 1) and 2 bp (g.7900-7901delCG, Figure 2) in the second and third exons, respec-

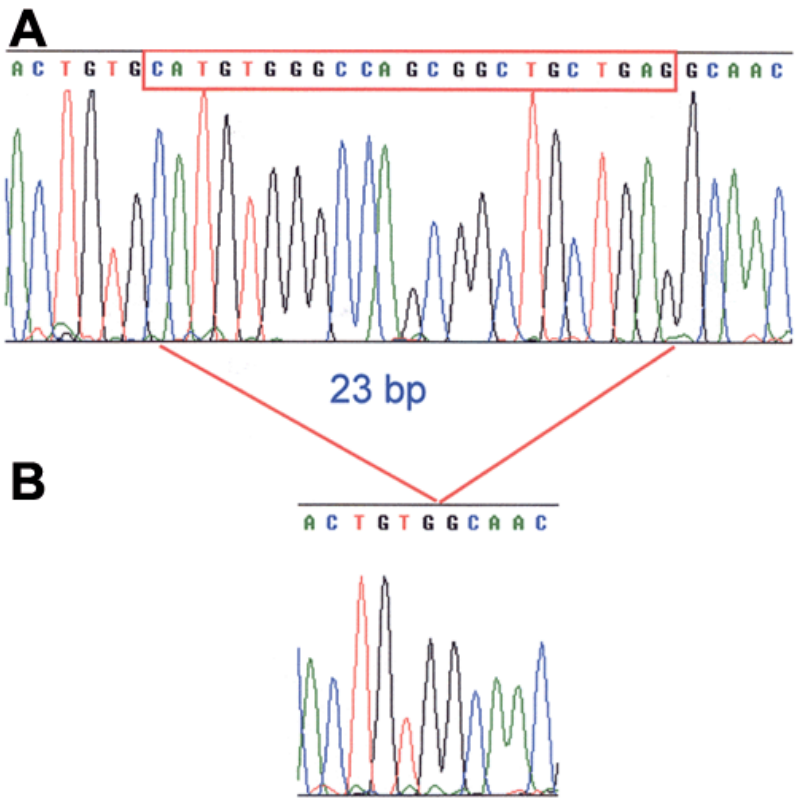

Figure 1. A novel mutation leading to the deletion of amino acid sequence HVGQRLLR in CYP1B1. Electropherogram of the sense strand of genomic DNA showing a homozygous deletion at g.3905-3927del23bp resulting in the inframe deletion of the 8 amino acids HVGQRLLR (**B**) compared to the sequence derived from the control (**A**).

tively. The deletions resulted in the creation of premature stop codons, thereby truncating the ORFs. The 23 bp deletion begins at nucleotide 3,905 and ends at 3,927 (reference sequence U56438) resulting in an inframe deletion of 8 amino acids i.e. the sequence his-val-gly-gln-arg-leu-leu-arg (or HVGQRLLR). Four novel missense mutations viz. g.4148G>C (A115P), g.4200T>G (M132R), g.4236A>C (Q144P), and g.4520A>C (S239R) were noted in the second exon, and one g.8234G>A (G466D) in the third exon (Figure 3). Except for S239R (that was present in 2 different cases), all other novel mutations were present in single cases only. All these mutations segregated with the affected phenotype and were absent in 200 normal chromosomes and their residues were mostly conserved across different species (Figure 4). Five of these mutations also resulted in the loss of restriction sites thereby facilitating their screening in the population (Table 1).

Other mutations in CYP1B1*:* Apart from these novel changes, 9 other mutations were noted in 16 PCG cases, which has already been reported in other ethnic groups [11,14,16,17,19-22]. These included a frameshift mutation leading to a 10 bp homozygous duplication g.8037-8046dupTCATGCCACC in PCG110 and 7 missense changes viz. L77P, R368H, R390H, R390C, P437L, E229K, and P193L (Table 1). The R368H was the most frequent mutant allele (7/25 cases) and also exhibited compound heterozygosity in association with R390C and G61E alleles in probands belonging to 2 non-consanguineous families. Three cases were heterozygous for the mutant alleles Q144P, R368H, and E229K. However no variation was observed in the non-coding regions or promoter of *CYP1B1* in the heterozygous cases indicating the involvement of some other causal gene that is yet uncharacterized.

We also observed six benign intragenic variants at -13T/C, R48G, A119S, V432L, D449D, and N453S that were reported in other populations [19]. But there was no common haplotype based on these single nucleotide polymorphisms that segregated with all these mutations, unlike in other populations [11,13,14].

DISCUSSION

Mutations in *CYP1B1* have been associated with PCG with varying frequencies across different ethnic communities and geographical boundaries [8,10-12]. Populations with higher

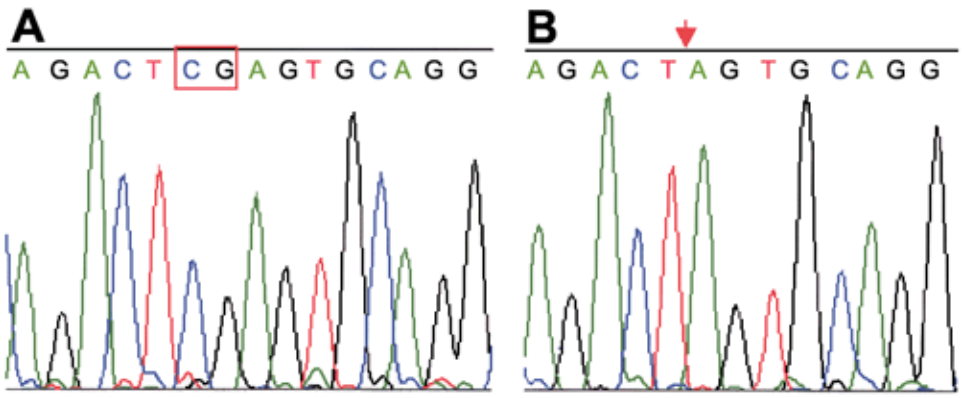

Figure 2. A novel deletion in CYP1B1. Electropherogram of the sense strand of genomic DNA showing a homozygous deletion at g.7900-7901delCG (**B**) compared to the sequence derived from normal control (**A**).

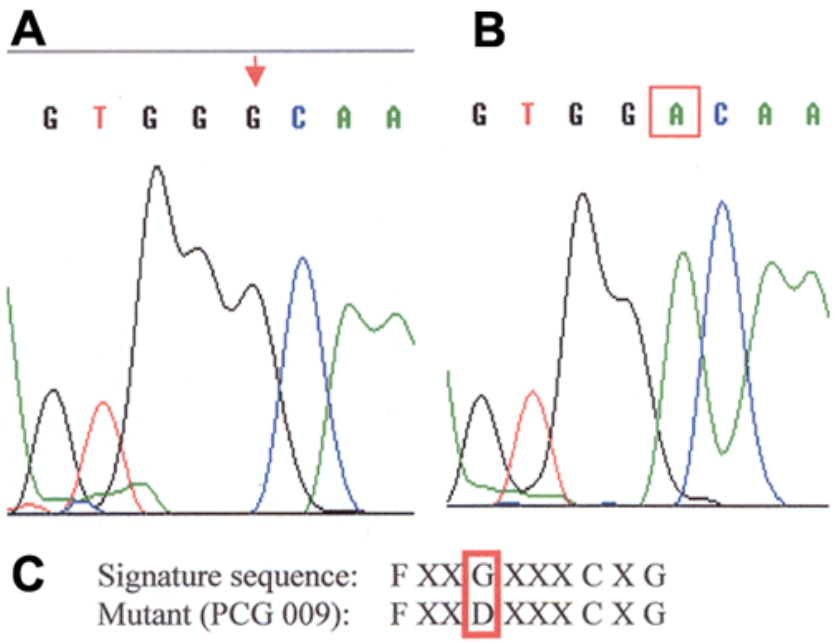

Figure 3. A novel mutation in the signature sequence of CYP1B1. Electropherogram of the sense strand of the genomic DNA showing a homozygous transition at g.8234G->A resulting in a G466D mutation (**B**) compared to the sequence derived from the normal control (**A**). Glycine forms a highly conserved part in the signature sequence of the gene (**C**).

rate of inbreeding and consanguinity exhibit a higher frequency of *CYP1B1* mutations in PCG as opposed to ethnically diverse populations [12,13]. Screening a cohort of 64 consecutive PCG cases from different geographical locales of India revealed 37.50% (24/64 cases) mutations in *CYP1B1*. Of these 24 cases, consanguinity was seen in 16 cases and 3 of them had a positive family history. Homozygosity of the mutant allele was seen in 29.68% (19/64) and compound heterozygosity in 3.12% (2/64) of the cases, respectively. However there was no association of any mutation with the gender or ethnicity of the individual.

The homozygous deletion at g.3905-3927delHVGQRLLR in the proband PCG050 (Figure 1) resulted in the truncation of the CYP1B1 protein to 51 aa, thereby missing 492 aa from the C-terminus. This occurred at the end of the membrane domain resulting in the elimination of all-important domains of CYP1B1 protein in exon II. It also led to the abolition of a restriction site for *Bbv*CI. The other homozygous deletion at g.7900-7901delCG in the proband PCG081 (Figure 2) resulted in the truncation of CYP1B1 protein at 373 aa in the "J" helix (350 aa-363 aa). The resulting protein therefore lacked the "K" and "L" helices along with the "heme binding" regions and also caused the loss of a restriction site for *Taq*I in exon III. The PCG050 and PCG 081 probands presented at 7 years and 2 months, respectively. Although surgical intervention could lower their IOPs, visual recovery was poor in both of them (Table 2).

Among the missense mutations, the homozygous substitution at g.4148G->C resulted in a change from alanine to proline (A115P) in exon II in the proband PCG008. This mutation occurred in the N-terminal region of the cytosolic domain between the "B" (100 aa-107 aa) and "C" (141 aa-151 aa) helices causing a loss of a restriction site for *Bsg*I. The importance of proline residues in the proline-rich region of microsomal cytochrome P450s has demonstrated the presence of typical carbon monoxide difference spectrum in the wild type P450 that was lacking in the mutant protein (due to the substitution of alanine by proline) [23]. Taking this into consideration, we suspect that the A115P mutation might also cause a conformational change of the protein due to an extra proline residue. This patient underwent a surgical intervention at 6 days after birth and had a better IOP control and visual recovery (Table 2).

Another homozygous substitution at g.4200T->G caused a change from methionine to arginine (M132R) in exon II in the proband and his younger brother in PCG122 family. This mutation was located between the "B" and "C" helices of the CYP1B1 protein that resulted in a loss of a restriction site for *Nla*III. A comparative modeling of CYP1B1 using CYP2C5 as a template showed the possibility of hydrogen bond formation with main chain amide in this residue (Unpublished). Thus this mutation could disrupt this hydrogen bond formation and affect the final conformation of the protein. Both the siblings in PCG122 family presented with raised IOP and visual acuity of "fixing and following of light" but the elder brother had to undergo Transcleralcyclophotocoagulation (TSCPC) due to his advanced nature of the glaucoma and late age of intervention (6 years).

A homozygous substitution at g.8234G->A resulted in the replacement of glycine by aspartic acid (G466D) in proband

P450 species	A115P	M132R	Q144P	S239R	G466D
Human CYP2A7	QAEEFSGRG	GYGVAF	RAKQLLRFA	TSTGQLYEM	SIGKR
Human CYP2B6	KAEAFSGRG	GYGVIF	RWKVLRRFS	SVFGQLFEL	SLGKR
Human CYP2F1	QGEEFSGRG	GNGIAF	RWKVLRQFS	SPWGELYDI	SAGRR
Human CYP2E1	YKDEFSGRG	DRGIIF	AWKDIRRFS	TPWLQLYNN	STGKR
Rat CYP1B1	QGGVFADRP	GRSLAF	RWKERRRAA	A--GSLVDV	SVGKR
Scup CYP1B1	QGGVFADRP	GRSLAF	RWKERRRAA	A--GSLVDV	SVGKR
Human CYP1B1	QGSAFADRP	GRSMAF	HWKVQRRAA	A--GSLVDV	SVGKR
Plaice CYP1B	QGTDFAGRP	GDSLAF	WWKVHRRVA	A--GSIVDV	SLGKR
Scup CYP1C2	HSTEFAGRP	GRSMTF	QWKMHRKIA	A--GSLVDV	SSGKR
Monkey CYP1A1	QGDDFKGRP	GWSMSF	VWAARRRLA	S--GNPADF	GLGKR
Human CYP1A1	QGDDFKGRP	GWSMSF	VWAARRRLA	S--GNPADF	GMGKR
Golden hamster CYP1A1	QGDDFKGRP	GKSMIF	VWAARRRLA	S--GYPPDF	GLGKR
Rabbit CYP1A2	QGDDFKGRP	GWSMIF	VWAARRRLA	S--GSPADF	GLGKR
Dog CYP1A2	QGDDFKGRP	SWSMSF	VWAAGRRLA	S--GNPLDF	GMGKR
Human CYP1A2	QGDDFKGRP	GWSLTF	VWAARRRLA	S--GNPLDF	GMGKR
Guinea pig CYP1A2	QSDDFKGRP	GWSMIF	VWAARRRLA	S--GNPVDF	GLGKR
Golden hamster CYP1A2	QGDDFKGRP	GKSMTF	VWAARRRLA	S--GNAVDF	GLGKR
Chicken CYPA4	QAEDFMGRP	GHSLAF	AWKARRKLA	A--GNPADF	GLGKR
Scup CYP1A1	QGDXFAGRP	GKSLAF	VWRARRKLA	S--GNPADF	GMGKR
Trout CYP1A1	QGEDFAGRS	GKSLAF	VWRARRKLA	S--GNPADF	GMGKR
Oyster toadfish CYP1A1	QGEDFSGRP	GKSLAF	VWRARRKLA	T--GNLADF	GLGKR
Human CYP17	KGKDFSGRP	NRKGIA	HWQLHRRLA	K--DSLVDL	GAGPR

Figure 4. Conservation of residues across cytochrome P450 families around the region of missense mutations. Multiple sequence alignment of Cytochrome P450 families showing the conservation of wild type amino acids (marked in red) for the 5 novel missense mutations. The human CYP1B1 protein is marked in blue.

PCG009 (Figure 3). This residue is highly conserved across all cytochrome P450s and is a part of the "signature sequence" (NH_2-FXX GXX XCX G-COOH) that is present in all heme binding cytochromes at the C-terminus of the protein (Figure 4). The cysteine residue in this sequence acts as the fifth ligand to heme. The other conserved residues and hydrophobic amino acids viz. phenylalanine and glycine next to the axial ligand (cysteine) are very important for the apoprotein to hold and/or incorporate the heme plane at the active site of P450 [24]. In spite of early intervention, visual prognosis was poor in this proband (Table 2).

Another homozygous mutation at g.4520A->C resulted in the replacement of serine by arginine (S239R) between the "F" (219 aa-234 aa) and "G" (259 aa-281 aa) helices of CYP1B1 protein. This was the only mutation that was observed independently in the probands of 2 families (PCG021, PCG147). Both the probands with S239R mutation had advanced glaucoma at presentation. Although their IOPs could be controlled by surgical intervention, their visual acuity did not improve significantly (Table 2).

A heterozygous change g.4236A->C resulted in the substitution of glutamine by proline (Q144P) in exon II in the proband PCG086. Although this residue is conserved only in humans (Figure 4), it was not seen in either of his parents or controls. This de novo mutation lay in the "C" helix (141 aa-151 aa) of the protein and caused a loss of a restriction site for *Msp*A1I. The insertion of proline in the middle of the "C" helix could induce a turn and further affect the final conformation of protein. This proband was intervened early and had a better prognosis with normal IOP and improved visual acuity (Table 2).

Among all the mutations, R368H was again found to be the most frequent change (29.16%), similar to our previous study that reported it to be the most predominant *CYP1B1* allele in the Indian population [17]. This mutation has rarely been reported from the other ethnic groups. So far only a few PCG families (from Saudi Arabia and Brazil) were found to have this mutation at a very low frequency [11,14]. Early intervention in 5 probands with the homozygous and heterozygous R368H mutant allele had a relatively better visual prog-

Table 1. Distribution of 16 *CYP1B1* mutations in 24 PCG patients in Indian and world populations

Serial number	Nucleotide change	Exon	Mutation	Restriction site	Cases (n=24)	Populations
1	g.3905-3927del23 bp	II		- Bbv CI	1	India
2	g.3987 G>A	II	G61E	+ Taq I	1*	Saudi Arabia [14] India
3	g.4035 T>C	II	L77P		1	Saudi Arabia [14] India
4	g.4148 G>C	II	A115P	- Bsg I	1	India
5	g.4200 T>G	II	M132R	- Nla III	1	India
6	g.4236 A>C	II	Q144P	- Msp A1I	1	India
7	g.4383 C>T	II	P193L	+ Eco 801I	1	India [16]
8	g.4491 G>A	II	E229K	- Eam 1140I	1	Lebanon [20] France [21] India [16]
9	g.4520 A>C	II	S239R		2	India
10	g.7900-7901delCG	III		- Taq I	1	India
11	g.7940 G>A	III	R368H	- Taa I	7	Saudi Arabia [14] Brazil [11] USA [22] India [16]
12	g.8005 C>T	III	R390C	+ Hinf I	2	India [17]
13	g.8006 G>A	III	R390H	- Cfo I	3*	Pakistan [19] India
14	g.8147 C>T	III	P437L		1	Turkey [19] Brazil [11] India
15	g.8234 G>A	III	G466D		1	India
16	g.8037-8046dupTCATGCCACC	III		+ Nla III	1	USA [22] UK [19] Turkey [19] Brazil [11] Germany [20] France [21] India

The details of 16 CYP1B1 mutations reported, along with their location in the gene, which exon, the type of change, and the restriction enzymes used for screening those mutations by RFLP. The "Cases" column indicates the number of cases in which the corresponding mutation was present out of a total 24 cases with mutations in the present study. The "Populations" column indicates the detection of a particular mutation in various global populations; references are cited for those noted reported herein. The minus sign (-) in the "Restriction site" column indicates the abolition of the restriction site and the plus sign (+) indicates the creation of the restriction site. In the "Cases" column, the asterisk indicates cases which include one compound heterozygous with R368H.

nosis, as opposed to probands who were compound heterozygous for R368H in association with G61E and R390H mutant alleles and intervened at later ages (Table 2). The codon 390 in *CYP1B1* happens to be a mutation hot spot in various populations [14,19,21]. However visual outcomes were relatively better in probands with the R390C mutation who underwent early surgical interventions than those with the R390H mutation, who were intervened late and also developed phthisis bulbi in one of their eyes (Table 2). Thus a variable prognosis was noted in probands with *CYP1B1* mutations. Although our clinical experience suggests a better visual prognosis for early intervention in PCG [25], our current findings may suggest a larger role of the type of mutation, leading to structural and functional alteration of the CYP1B1 protein. However, functional studies of these mutations and analysis on a larger series would throw more light in understanding the protein alterations leading to the severity of phenotype in PCG.

The involvement of *CYP1B1* in PCG was found to be lower (37.50%) in our population than those reported in Saudi Arabian (95.0%) and Slovakian Gypsy populations (100%). This could be due to a higher rate of inbreeding and allelic homogeneity in these populations [12,13]. Relatively lower frequencies of *CYP1B1* mutations have been observed in Brazilian (50%) and south east Asian populations such as Japanese (20%) and Indonesians (about 34%) [8,10,11]. Interestingly, none of the *CYP1B1* mutations in Japanese and Indonesian populations were found in Indian PCG populations. This could be attributed to the genetic heterogeneity and ethnic diversities in these populations. As there was no association of any specific intragenic haplotypes to these mutations, it could imply multiple independent origins of these mutations that warrants further investigations. In summary, this study provides a mutation spectrum of *CYP1B1* causing PCG in Indian populations that has implications in devising molecular diagnostics for rapid screening in predisposed families [22] and would aid early intervention.

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Table 2. Clinical features of 24 PCG probands with *CYP1B1* mutations

Patient ID	Mutation	Alleles	Marriage	Age at onset	Age at diagnosis	Final IOP		Final Acuity	
						OD	OS	OD	OS
PCG050	g.3905-3927del23 bp	H	C	birth	7 year	24	30	CF (1m)	LPP
PCG107	L77P	H	C	birth	18 year	26	20	LPP	LPP
PCG008	A115P	H	C	birth	6 day	18	16	20/40	20/30
PCG122	M132R	H	NC	2 month	6 year	NA	22	NLP	20/1200
PCG086	Q144P	h	NC	birth	10 day	8	10	20/130	20/130
PCG131	P193L	H	NC	birth	16 month	11	13	20/170	20/170
PCG155	E229K	h	C	birth	2 month	12	12	20/50	20/50
PCG021	S239R	H	C	birth	4 year	19	10	20/125	NLP
PCG147	S239R	H	C	birth	3 month	10	20	FFL	FFL
PCG081	g.7900-7901delCG	H	C	birth	2 month	18	24	FFL	FFL
PCG171	R368H	H	NC	birth	2 year	12	NA	FFL	FFL
PCG185	R368H	H	C	birth	18 day	24	24	FFL	FFL
PCG186	R368H	H	C	birth	27 day	16	18	20/1900	20/1900
PCG189	R368H	H	C	birth	1 month	12	14	20/63	20/63
PCG182	R368H	h	NC	birth	6 day	14	14	20/130	20/130
PCG120	G61E, R368H	CH	NC	1 month	9 year	18	34	20/20	HM
PCG190	R368H, R390H	CH	NC	birth	16 year	38	12	20/100	20/30
PCG180	R390C	H	C	birth	1 Day	12	10	20/470	20/470
PCG184	R390C	H	NC	birth	20 day	12	16	20/470	20/470
PCG154	R390H	H	C	15 day	4 year	NA	21	NLP	LPP
PCG119	R390H	H	C	birth	10 year	27	NA	20/125	NLP
PCG094	P437L	H	C	birth	10 day	26	24	20/200	LPP
PCG009	G466D	H	C	birth	2 month	15	NA	20/760	NLP
PCG110	g.8037-8046dupTCATGCCACC	H	C	birth	2 year	16	26	FFL	FFL

Details of clinical features of the 24 probands with CYP1B1 mutations indicating their genotype, age at onset, and diagnosis along with their visual outcomes following surgical intervention with respect to IOP and visual acuity at the last follow-up. The “Alleles” column indicates whether the patient was homozygous (H), heterozygous (h), or compound heterozygous (CH). Marriages were classified as consanguineous (C) or non-consanguineous (NC). The final Intraocular pressure (IOP) is given except when it was not available due to a phthisical eye. The final visual acuity is given as a snellen measurement when possible; otherwise, it is listed as hand movements (HM), counts fingers (CF), light perception with projection (LPP), fixes and follows light (FFL), and no light perception (NLP).

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