CYP1B1 Mutation Profile of Iranian Primary Congenital Glaucoma Patients and Associated Haplotypes

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The mutation spectrum of *CYP1B1* among 104 primary congenital glaucoma patients of the genetically heterogeneous Iranian population was investigated by sequencing. We also determined intragenic single nucleotide polymorphism (SNP) haplotypes associated with the mutations and compared these with haplotypes of other populations. Finally, the frequency distribution of the haplotypes was compared among primary congenital glaucoma patients with and without *CYP1B1* mutations and normal controls. Genotype classification of six high-frequency SNPs was performed using the PHASE 2.0 software. *CYP1B1* mutations in the Iranian patients were very heterogeneous. Nineteen nonconservative mutations associated with disease, and 10 variations not associated with disease were identified. Ten mutations and three variations not associated with disease were novel. The 13 novel variations make a notable contribution to the ~70 known variations in the gene. *CYP1B1* mutations were identified in 70% of the patients. The four most common mutations were G61E, R368H, R390H, and R469W, which together constituted 76.2% of the *CYP1B1* mutated alleles found. Six unique core SNP haplotypes were identified, four of which were common to the patients with and without *CYP1B1* mutations and controls studied. Three SNP blocks determined the haplotypes. Comparison of haplotypes with those of other populations suggests a common origin for many of the mutations.

Glaucoma is a heterogeneous group of optic neuropathies with common manifestations including a specific pattern of visual field loss and degeneration of the optic nerve resulting in a characteristic glaucomatous appearance.[1,2] Degeneration of the optic nerve may be caused by apoptosis of retinal ganglion cells.[3] Glaucoma leads to blindness if left untreated, and it is considered the second leading cause of blindness worldwide.[4] The disease is subclassified based on etiology, anatomy of the anterior chamber of the eye, and age of onset.[2] The subgroup primary congenital glaucoma (PCG; Online Mendelian Inheritance of Man no. 231300) is a severe form of the disease. It is characterized by an anatomical defect of the trabecular meshwork (trabeculodysgenesis) and an age of onset in the neonatal or infantile period, generally before the age of 3 years.[5] The developmental anomaly at the angle of the anterior chamber manifests itself by increased intraocular pressure (IOP), corneal edema, excessive tearing, photophobia, enlargement of the globe (buphthalmos), and opacity of the cornea. The details of the pathogenic pathways, including the relationship between elevated IOP and optic nerve damage,

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are not well understood. PCG occurs in both sporadic and familial patterns. In familial cases, inheritance is usually autosomal recessive, sometimes associated with incomplete penetrance.[5,6] Pseudodominant transmission has also been reported.[7] The incidence of PCG is geographically and ethnically variable, estimated at 1:10,000 in Western countries[5] and higher in inbred populations such as those of Andhra Pradesh in India (1:3300),[8] Saudi Arabia (1:2500),[6] Slovakia Roma (1:1250),[9] and Arab Bedouins of the Negro region in Israel (1 of 1200).[10]

So far, three PCG loci have been identified by linkage analysis in multiply affected families, GLC3A,[11] GLC3B,[12] and GLC3C.[5,13] Only the gene associated with GLC3A, *CYP1B1* (Online Mendelian Inheritance of Man no. 601771), has been identified.[14] The *CYP1B1* gene spans ~12 kb on chromosome 2, has three exons, encodes cytochrome P4501B1, and is a member of the cytochrome P450 superfamily of genes.[15] Protein products of these genes catalyze oxidative, peroxidative, and reductive reactions and have roles in the metabolism of various substrates. Expression of the *CYP1* gene family, which includes *CYP1B1*, is induced by the aryl hydrocarbon receptor. Although physiological studies have confirmed that mutations in *CYP1B1* can cause disease, the pathway by which *CYP1B1* affects development of the anterior chamber of the eye is unknown.[15,16] Presumably, the metabolism of an endogenous substrate by the CYP1B1 protein is involved. Expression of *CYP1B1* in the posterior segment of the eye, notably in the neuroretina, may be relevant to glaucoma pathogenesis.[17] In addition to glaucoma, *CYP1B1* may have a role in carcinogenesis. Unusually high expression of the gene or increased frequencies of alleles coding more active isoforms have been reported in some cancers.[18–20]

The proportion of PCG patients whose disease is attributable to *CYP1B1* mutations is generally high but varies among populations. Comparisons are not definitive, particularly because of differences in sample size, composition of samples with regard to familial and sporadic classifications, and detection protocols; nevertheless, the published figures clearly indicate that the variation exists. The numbers range from 100 to 20%: 100% in Slovakia Roma,[9] ~90% in Saudi Arabia,[6] ~50% in Brazil[21] and France,[22] ~40% in India[23] and Morocco,[24] and ~20% in Japan.[25] *CYP1B1* may have a lesser role in the disease status of African PCG patients as compared with Europeans.[21]

The worldwide profile of variations thus far reported is heterogeneous and includes ~70 alterations (Human Genome Mutation Database; *http://www.hgmd.cf.ac.uk/ac/index.php*). The degree of heterogeneity within different populations, as well as the distribution of mutations, is quite variable.[26] A single allele, E387K, constitutes all *CYP1B1* mutated alleles among the Slovak Roma patients.[9] Likewise, only the V364M mutation was found among PCG patients of Indonesian descent.[27] In Saudi Arabia, G61E constitutes ~75% of the mutated alleles, and R469W and D374N account for almost all of the rest.[6,28] Mutation g.4339delG is the predominant mutation among patients from Morocco.[24] Among less than 30 PCG patients with *CYP1B1* mutations from India[23] and Brazil,[21] respectively, 16 and 11 different mutations were found. However, a single mutation, R368H in India and g.4340delG in Brazil, constituted ~20% of the aberrant alleles in their respective populations. In contrast to these populations, 11 different mutations were found among only eight patients of French descent carrying *CYP1B1* mutations.[22] The same number of mutations was identified among 13 Japanese patients.[25] These differences are likely attributable to variations in frequencies of consanguineous marriages and gene pools among the different populations.

The genetic basis of PCG among Iranian patients has not been previously studied. Iran, having been a major gateway in human history, has encountered many populations and is expected to have a rich genetic legacy. Here, we report the frequency of Iranian PCG patients carrying mutations in the coding regions of the *CYP1B1* gene. Mutations thought to be associated with PCG, including 10 novel ones, and variations thought not to be associated with PCG, three of which are novel, are described. Intragenic single nucleotide polymorphism (SNP) haplotypes associated with the mutations are presented and compared with those previously reported for other populations.

Materials and Methods

This research was performed in accordance with the Helsinki Declaration and with the approval of the ethics board of the International Institute for Genetic Engineering and Biotechnology in Iran. The families of patients all consented to participate after being informed of the nature of the research. One hundred four unrelated patients were recruited mostly from the ophthalmic divisions of the Farabi (associated with Tehran University of Medical Sciences), Labbafi-Nejhad (associated with Shaheed Beheshti University of Medical Sciences and Health Services), and Hazrat Rasoolakram (associated with Iran University of Medical Sciences) hospitals in Tehran. The hospitals are national reference centers, and patients from throughout the country are referred to them. All patients were diagnosed by glaucoma specialists. Slit lamp biomicroscopy, measurement of IPO, gonioscopy (if corneal clarity permitted), fundus examination, and measurement of perimetry were performed whenever possible. IOP measurements were obtained using Goldmann tonometry or the Tono-Pen (Medtronic, Minneapolis, MN) in cases with limited cooperation or central corneal scars. PCG manifested in the patients by IOP of ≥21-mm mercury (21 to 56 mm Hg) in at least one eye before treatment, corneal edema, Descemet membrane rupture, megalocornea (corneal diameter >12 mm), and optic nerve head changes suggestive of glaucomatous damage including high cup/disc ratio or neuronal rim thinning or notching. The cup/disc ratio of affected eyes when available ranged from 0.3 to total cupping (average, 5.8). Patients with other ocular or systemic anomalies were excluded. For example, patients diagnosed with Peters' anomaly or aphakic glaucoma after congenital cataract surgery were not included. Age of onset ranged

from birth to 3 years. One hundred sixty ethnically matched but unrelated control individuals were recruited from those older than 60 years of age and without self-reported familial history of ocular diseases. Older individuals were recruited because mutations in *CYP1B1* has been reported in some late onset glaucoma patients.[22,29]

The patients were recruited consecutively, without regard to familial status of disease. Of the 104 PCG patients, 33 were sporadic in the sense that their parents indicated no consanguinity and no other incidence of disease in relatives of the patient. Fifty-eight patients were offspring of consanguineous parents. Of these, 46 had no other affected family member. Seventeen patients were recurrent cases in the sense that more than one family member was affected with PCG. Five of these were progeny of reported nonconsanguineous marriages. PCG in progeny of consanguineous marriages and in recurrent cases was considered familial; there were thus 63 familial PCG patients. The sporadic/familial status of eight patients could not be ascertained.

Exon 1 of the *CYP1B1* gene was amplified by polymerase chain reaction (PCR) in 50 patients. The primers corresponded to sequences adjacent to the exon (F, forward; R, reverse) (1F: 5′-GAAAGCCTGCTGGTAGAGCTCC-3′; 1R: 5′-CTGCAATCTGGGGACAACGCTG-3′). Exon 2, which contains the initiation codon, was amplified in all 104 patients in two overlapping PCR fragments (2Fa: 5′-ATTTCTCCAGAGAGTCAGCTCCG-3′; 2Ra: 5′-TGTAGCGGCAGCCGAAACACAC-3′; Fb: 5′-GCATGATGCGCAACTTCTTCACG-3′; 2Rb: 5′-TCACTGTGAGTCCCTTTACCGAC-3′). The coding region of exon 3 was also amplified in all of the patients (3F: 5′-AATTTAGTCACTGAGCTAGATAGCC-3′; 3R: 5′-TATGGAGCACACCTCACCTGATG-3′). The amplicon of exon 1 included 172 nucleotides upstream of the transcriptional initiation site and 136 nucleotides of intron 1. The amplicons of exon 2 included 133 and 161 nucleotides of introns 1 and 2, respectively. The amplicon of exon 3 included 129 nucleotides of intron 2 and 155 nucleotides downstream of its protein coding region. All PCR products were sequenced in both forward and reverse directions with the same primers as used in the PCRs, using the ABI Big Dye terminator chemistry and an ABI Prism 3700 instrument (Applied Biosystems, Foster City, CA). The *CYP1B1* amplicons of 10 control individuals were also fully sequenced. Sequences were analyzed by the Sequencher software (Gene Codes Corp., Ann Arbor, MI).

Four of the novel single nucleotide variations deemed to be possibly associated with disease were assessed in 60 to 109 control individuals by restriction enzyme digestion and fragment length polymorphism (RFLP) as described below. All of the controls were Iranian, and at least 50 were from the same region of the country as the patients carrying the variations. Likewise, five SNPs contributing to unique core haplotypes were assessed in 100 control individuals from throughout Iran by RFLP. The enzymes used for g.3947C>G (R48G), g.4160G>T (A119S), g.8131G>C (V432L), g.8184T>C (D449D), and g.8195A>G (N453S) were *Bsa*WI, *Nae*I, *Ale*I, *Bse*GI, and *Mwo*I, respectively. The restriction enzymes were purchased from New England BioLabs (Boston, MA), Roche (Mannheim, Germany), or Cinnagen (Tehran, Iran). One SNP of the haplotypes of the controls, g.3793T>C, was assessed only in the 10 individuals sequenced because a restriction enzyme appropriate for its analysis by RFLP was not identified.

Unique core haplotypes consisting of six SNPs were assessed in patients with *CYP1B1* mutations, patients without *CYP1B1* mutations, and the control group using the PHASE 2.0 software.[30,31] This program of the software implements a Bayesian statistical method for reconstructing haplotypes. Use of the fastPHASE 1.0.1 software, which is based on a cluster model that includes an E-M algorithm, produced identical results.[32] Statistical comparisons of haplotype frequencies between and among groups were done using χ^2 contingency tables.[33]

Sequence variations and numbering were assessed by comparison with reference sequences associated with *CYP1B1* available at National Center for Biotechnology Information (*http://www.ncbi.nlm.nih.gov*; genomic sequence, NT_022184.14; cDNA sequence, NM_000104.2, protein sequence, NP_000095.1). Predicted effects of variant sequences on splicing were determined by comparison with known canonical splice site motifs (*http://www.fruitfly.org/seq_tools/splice.html*). For determination of extent of conservation of amino acids altered because of nucleotide variations, the amino acid sequences of 34 cytochrome P450 proteins from 18 species were obtained from SwissProt (*http://expasy.org./sprot/*) and aligned using the ClustalW software (European Bioinformatics Institute, Hinxton, UK; *http://www.ebi.ac.uk/clustalw*).

Results

Novel Variations

Twenty-nine sequence variations were identified in the regions of the *CYP1B1* gene sequenced in DNA of the Iranian PCG patients and controls (Tables 1 and 2). Ten of the variations have been previously reported to be mutations associated with disease in the literature and six others reported as variations thought not to cause PCG; most of these are listed in the Human Genome Mutation Database. Although *CYP1B1* polymorphisms and mutations associated with PCG have now been reported in various ethnic groups, only limited information is available on genotype-phenotype correlations.[21,34–36] Among the previously reported disease-causing mutations also found among the Iranian patients, some phenotypic data for T404fs (g.8037-8096dup10) in the Brazilian population are available.[21] The phenotypic features of two Brazilian homozygotes carrying this mutation were similar to those of the single homozygous Iranian patient: the three were diagnosed before the age of 1 month, both eyes were affected in all, and their maximum recorded IOP ranged from 26 to 31.5 mm Hg. Phenotypic features associated with mutations found among a larger number of both Iranian and Indian patients are presented in Table 3.[35] Only data on homozygous patients are presented so as to eliminate variations attributable to effects of differing second mutations. Data on R390H and R469W are in-

Table 1. *CYP1B1* Mutations Associated with PCG in Iranian Patients

Gene location*	cDNA location*†	Exon	Effect on protein*	Nature of amino acid change‡	Number of patients		Total no. alleles	Percent of PCG causing chromosomes§	Percent of mutated CYP1B1 alleles
					Hom	Het			
g.3987G>A	**c.182G>A**	2	**p.G61E**	NC	15	15	45	21.6%	32.4%
g.3988delA¶	c.183delA¶	2	p.G61fs¶		1	0	2	1.0%	1.4%
g.4048C>A¶	c.243C>A¶	2	p.Y81X¶		1	0	2	1.0%	1.4%
g.4322G>A	c.517G>A	2	p.E173K	NC	2	0	4	1.9%	2.9%
g.4410C>A¶	c.620C>A¶	2	p.A202D¶	NC	0	1	1	0.5%	0.7%
g.4490G>A	c.685G>A	2	E229K	NC	1	1	3	1.4%	2.2%
g.4611_4619 dupGCAACTTCA¶	c.806–814 dupGCAACTTCA¶	2	p.N265_R266 insSNL¶		1	0	2	1.0%	1.4%
g.4673_4674insC	c.862insC	2	p.R290fs		2	0	4	1.9%	2.9%
g.4677A>G¶	c.872A>G¶	2	p.D291G¶	NC	1	0	2	1.0%	1.4%
g.4791G>T¶	c.986G>T¶	2	p.G329V¶	NC	0	2	2	1.0%	1.4%
g.7934delG¶	c.1097delG¶	3	p.R366fs¶		1	0	2	1.0%	1.4%
g.7939C>T¶	c. 1102C>T¶	3	p.R368C¶	NC	1	1	3	1.4%	2.2%
g.7940G>A	**c. 1103G>A**	3	**p.R368H**	NC	2	9	13	6.3%	9.4%
g.8006G>A	**c. 1169G>A**	3	**p.R390H**	NC	12	8	32	15.4%	23.0%
g.8037_8046 dupTCATGCCACC	c.1200_1209 dupTCATGCCACC	3	p.T404fs		1	1	3	1.4%	2.2%
g.8162C>G	c.1325C>G	3	p.P442R	NC	0	1	1	0.5%	0.7%
g.8242C>T	**c.1405C>T**	3	**p.R469W**	NC	7	2	16	7.7%	11.5%
g.8341delA¶	c.1504 del A¶	3	p.M503fs¶		0	1	1	0.5%	0.7%
g.8354_8373del GTTATGGTCT-AACCATTAAA¶	c.1517_1536 delGTTATGGT-CTAACCATTAAA¶	3	p.S506fs¶		1	0	2	1.0%	1.4%
								67.5%	~100%

*The four most common mutations are shown in bold. Reference sequences used were NT_022184.14, NM_000104.2, and NP_000095.1.
†A of the initiation codon was designated +1.
‡Based on biochemical properties of size and charge.
§Assuming autosomal recessive status of disease in all patients.
¶Novel mutation.
Hom, homozygous; Het, heterozygous; fs, frameshift: NC, nonconservative.

cluded because these are common mutations among the Iranian patients. The data on R390H of the Iranians also allow comparison with R390C, which affects the same amino acid position and is found among the Indian patients. The remaining 13 novel variations were designated mutations associated with PCG or variations probably not associated with the disease on the basis of causing frameshifts or creating stop codons during translation, absence in control individuals, presence in more than one unrelated patient, occurring at same site as a previously reported mutation, nature of amino acid change caused, and/or degree of conservation during evolution.

Variations g.3988delA(G61fs), g.7934delG(R366fs), g.8341delA(M503fs), and g.8354_8373delGTTATGGTCT-AACCATTAAA(S506fs) were considered as pathogenic because they all caused frameshifts (Table 1). The Y81X alteration was classified as disease-causing because it results in very early truncation of the CYP1B1 protein. Variations A202D, D291G, G329V, and R368C were also considered putative disease-causing mutations. The nucleotide substitutions causing these amino

Table 2. *CYP1B1* Variations Not Associated with PCG in Iranian Patients

Gene location	cDNA location*	Exon	Effect on protein	Nature of amino acid change†	Number of patients		Total no. alleles	Minor allele frequency	Reference SNP number‡
					Hom	Het			
g.3318 insC	5′NC.-487insC	2			50	0	100	0%	
g.3793 T>C	IVS I.-13T>C	2			17	21	55	27.0%	rs4987134
g.3947 C>G	c.142C>G	2	p.R48G	NC	17	21	55	27.0%	rs1001
g.4131 A>G§	c.326A>G§	2	p.Q109R§	C	1	0	2	1.0%	
g.4160 G>T	c.355G>T	2	p.A119S	C	17	21	55	27.0%	rs1056827
g.4612 C>T§¶	c.807C>T§¶	2	p.S269S§¶	S	0	1	1	0.5%	
g.8032 A>G§	c.1195A>G§	3	p.I399V§	C	1	0	2	1.0%	
g.8131 G>C	c.1294G>C	3	p.V432L	C	39	25	103	49.5%	rs1056836
g.8184 T>C	c.1347T>C	3	p.D449D	S	39	25	103	49.5%	rs1056837
g.8195 A>G	c.1358A>G	3	p.N453S	C	8	10	26	12.5%	rs1800440

*A of the initiation codon was designated +1.
†Based on biochemical properties of size and charge.
‡From build 125 of the SNP database at the National Center for Biotechnology Information.
§Novel variation.
¶This variation was found in a control individual.
Hom, homozygous; Het, heterozygous; NC, nonconservative; C, conservative; S, synonymous.

Table 3. Phenotype-Genotype Correlations in PCG Patients of Iran and India

	Onset by birth[†]		Corneal diameter (mm) [‡]		IOP (mm Hg)[‡]		C/D ratio[‡§]	
Mutation*	India	Iran	India	Iran	India[¶]	Iran[‖]	India	Iran
G61E	3 (3)	9 (15)	3.9 (6)	13.4 (29)	30.2 (6)	29.1 (28)	0.48 (4)	0.49 (15)
R368H	17 (19)	1 (2)	13.0 (38)	15.3 (3)	26.1 (36)	29.7 (3)	0.48 (20)	0.87 (3)
R390C	5 (5)		12.5 (8)		26.6 (10)		0.30 (2)	
R390H		5 (8)		12.7 (24)		33.4 (24)		0.65 (8)
R469W		2 (3)		13.9 (8)		30.0 (14)		0.60 (6)

Data for patients from India based on Ref. 35.
*All homozygous.
[†]Number of patients (no. of patients with the mutation).
[‡]Average of eyes for which data is available (no. of eyes for which data is available).
[§]Cup/disc ratio of the optic nerve.
[¶]IOP at diagnosis.
[‖]Maximum IOP.

acid changes were not found in the DNA of control individuals by RFLP analysis (not shown). All result in nonconservative amino acid alterations with respect to size and charge at positions where the wild-type residue is highly conserved (Table 4). In addition, D291G lies within a –PGAARDM– sequence and G329V within a 15-amino acid sequence (–TDIFGASQDTLSTAL–) in helix I common to CYP1B1 sequences of distally related species (Table 4). G329V and R368C were observed in more than one patient. Furthermore, R368C affects the same amino acid position as R368H, which has been reported by others to be associated with disease.[34,37] Finally, g.4611_4619dupGCAACTTCA causes an in-frame insertion of Ser/Asn/Leu after residue 265 and was considered a disease-causing mutation. This 9-bp sequence is tandemly repeated twice in the *CYP1B1* reference sequence NT_022184.14. Deletion of one of the repeats has previously been reported as a deleterious mutation.[6] Furthermore, the insertion disrupts the –NRNFS– sequence that is highly conserved among CYP1B1s of species as distally related as the dolphin and human (not shown).

Table 4. Alignment of Novel Amino Acid Variations in Cytochrome P450 Proteins

Variation	Q109R	A202D	D291G	G329V	R368C	I399V	Seq ID*
CYP1A1_Human	QALVRQGD	VVSVTNVIC	EK-GHIRDIT	IVLDLFGAGFDTVTTAIS	RKIQEELDTVIGRSRRPRLS	VPFTIPHST	sp P04798
CYP1A1_Mouse	QALVRQGD	VVSVANVIC	EK-GHIRDIT	IVLDLFGAGFDTVTTAIS	RKIQEELDTVIGRDRQPRLS	VPFTIPHST	sp P00184
CYP1A1_Rat	QALVKQGD	VVSVANVIC	EK-GHIRDIT	IVFDLFGAGFDTITTAIS	RKIQEELDTVIGRDRQPRLS	VPFTIPHST	sp P00185
CYP1A1_Monkey	QALVQQGD	VISVANVIC	EK-GHIRDIT	VVLDLFGAGFDTVTTAIS	RKIQEELDTVIGRSRRPRLS	VPFTIPHST	sp Q6GUR1
CYP1A1_Dog	QALVRQGD	VVSVANVIC	EK-GQIRDVT	VVLDLFGAGFDTVTTAIS	KKIQKELDTVIGRARQPRLS	VPFTIPHST	sp P56590
CYP1A1_Sheep	QALVRQGD	VVSVANVIC	EK-GHIRDIT	VVMDLFGAGFDTVTTAIS	KKIQEELDTVIGRARWPQLS	VPFTIPHST	sp P56591
CYP1A1_Guinea pig	QALVRQGD	VVSVANVIS	EK-GHIRDIT	IVLDLFGAGFDTITTAIS	KKIQEELDTVIGRERQPQLA	MPFTIPHST	sp Q06367
CYP1A1_Hamster	QALVRQGD	VVSVTNVIC	EK-GHIRDIT	IIVDLFGAGFDTVTTAIS	RKIQEELDTVIGRSRRPRLC	LPFTIPHST	sp Q00557
CYP1A1_Rabbit	QALVRQGD	VMSVANVIC	EK-GHIRDIT	IVLDLFGAGFDTVTTAIS	RKIQEELDAVVGRARRPRFS	LPFTIPHST	sp P05176
CYP1A1_Sea bream	QALIKQGD	VVSVANVIC	DK-DNIRDIT	IVNDLFGAGFDTISTALS	ERLYQEMKESVGLDRTPCLS	LPFTIPHCS	sp O42457
CYP1A1_Scup	QALIKQGD	VVSVANVIC	DK-DNIRDIT	IVNDLFGAGFDTISTALS	ERLYQEMNETVGPDRTPCLS	LPFTIPHCT	sp Q92116
CYP1A1_Plaice	QALIKQGD	VVSVANVIC	NK-DNIRDIT	IVNDLFGAGFDTVSTALS	ERLYQEIEDKVGLDRMPLLS	LPFTIPHCT	sp Q92100
CYP1A1_Oyster	QALIKQGE	VVSVANVIC	NK-DNIRDIT	IVNDLFGAGFDTVSTGLS	ERLYQEIKDSVGTERMPLLS	LPFTIPHCT	sp Q92095
CYP1A1_Trout	QALIKQGE	VVSVANVIC	DK-DNIRDIT	IVNDLFGAGFDTISTALS	ERLHQELKEKVGMIRTPRLS	LPFTIPHCT	sp Q92110
CYP1A1_Tomcod	QALIKQGH	VVSVANVIC	DK-DNIRDIT	IVNDLFGAGFDTVSTALS	ERLHQEIKDKVGLSRSPVLT	LPFTIPHCA	sp Q92148
CYP1A2_Human	QALVRQGD	VVSVANVIG	DK-NSVRDIT	LVNDIFGAGFDTVTTAIS	RKIQKELDTVIGRERRPRLS	LPFTIPHST	sp P05177
CYP1A2_Dog	QALVRQGD	LLSVANVIG	DE-RSVQDIT	LINDIFGAGFDTVTTAIS	RQIQKELDTVIGRARQPRLS	VPFTIPHST	sp P56592
CYP1A2_Rabbit	QALVRQGD	VVSAARVIG	DR-NSIQDIT	LVNDIFGAGFDTITTALS	RKIQEELDAVVGRARQPRLS	VPFTIPHST	sp P00187
CYP1A2_Mouse	QALVRQGD	VESVANVIG	NK-NSIQDIT	IVNDIFGAGFDTVTTAIT	RKIHEELDTVVGRDRQPRLS	VPFTIPHST	sp P00186
CYP1A2_Rat	QALVKQGD	VESVANVIG	NK-NSIQDIT	IVNDIFGAGFETVTTAIF	RKIHEELDTVIGRDRQPRLS	VPFTIPHST	sp P04799
CYP1A2_Hamster	QALVRQGD	VESVANVIG	NK-NSIQDIT	IVNDLFGAGFDTVTTAIT	RKIHKELDTVIGRDRQPRLS	VPFTIPHST	sp P24453
CYP1A2_Guinea pig	QALVRQSD	VGSVANVIG	DK-NHVQDIA	LVNDIFGAGFDTVTTAIS	KKIHKELDAVIGRDRKPRLA	LPFTIPHCT	sp Q64391
CYP1A3_Trout	QALIKQGE	VVSVANVIC	DK-DNIRDIT	IVNDLFGAGFDTISTALS	ERLHQELKEKVGMIRTPRLS	LPFTIPHCT	sp Q92109
CYP1A4_Chicken	QALVRQAE	MVSVANVIC	DK-EHIRDVT	IVNDLFGAGFDTVTTALS	KKIQAELDQTIGRERRPRLS	LPFTIPHCT	sp P79760
CYP1A5_Chicken	QALVRQAE	VVSVANVIC	DK-NNIRDVT	LVNDIFGAGFDTVTTALS	KKIQAELDQTIGRERRPRLS	MPFTIPHST	sp P79761
CYP1B1_Human	QALVQQGS	VVAVANVMS	RPGAAPRDMM	TITDIFGASQDTLSTALQ	TRVQAELDQVVGRDRLPCMG	VPVTIPHAT	sp Q16678
CYP1B1_Mouse	QALVQQGS	IVAVANVMS	VPGAAPRDMT	TITDIFGASQDTLSTALL	ARVQAELDQVVGRDRLPCMS	LPVTIPHAT	sp Q64429
CYP1B1_Rat	QALVQQGG	IVAVANVMS	VPGAAPRDMM	TITDIFGASQDTLSTALL	ARVQAELDQVVGRDRLPCMS	LPVTLPHAT	sp Q64678
CYP1B1_Dolphin	--------	-----NVMS	RPGAAPRDMM	TVTDIFGASQDTLSTALQ	ARVQAELDQVVGRDRLPCLD	VPVTIPHAT	tr Q8SQH0
CYP1B_Plaice	QALVKQGT	VVSTANIMS	QS-STTRDMT	TMGDIFGASQDTLSTALQ	LRIQQEVDKVVDRTRLPSIE	VPLTIPHST	tr Q9W713
CYP1B_Zebrafish	--------	---------	----------	--------SQDTLSTALQ	KRLQEDVDRVVDRSRLPTIA	TPLTIPHST	tr Q8QFQ1
CYP1C1_Scup	EALIQHST	TVAAANIMC	DP-EVTRDMS	TVTDLIGAGQDTVSTVMQ	AKLQELIDKVVGQDRLPSIE	VPVTIPHST	tr Q8QGR5
CYP1C2_Scup	EALIQHST	TVAAANVIC	DP-EVTRDIS	TVSDLIGAGLDTVSTALH	TKLHELIDKVVGRQRLPSIE	VPVTIPHST	tr Q8QGR1
CYP2V1_Zebrafish	KVLNDQGN	NNGVSNIIC	DP-SSPRDFI	AVLDLFVAGTETTSTTLL	EKVQAEIDKVVGRYRRPSMD	VPLSVPRMT	tr Q4L203

*Sequence ID numbers at Expasy server (*http://www.expasy.org/sprot/*).

Table 5. Phenotype of PCG Patients with Novel *CYP1B1* Mutations

Mutation	Hom/Cpd Het	Fam/ Sp*	Age of onset	Effected eye	C/D ratio R/L	Corneal opacity† R/L	Edema R/L	Megalo-cornea‡ R/L	Haab's striaie§	IOP Max (mm Hg) R/L	Surgery (trabec-ulotomy)
G61fs	Hom	F	Birth	Bilateral		++/++	+/+			28/28	Multiple
Y81X	Hom	F	Birth	Bilateral		++/++	+/+	+/+	+		3×
A202D	Het(2^{nd} mut G61E)	S	6 months	Bilateral	En/0.6	En/−	+/+	En/		En/36	Multiple
N265ins SNL	Hom	?		Bilateral	0.6/0.6	++/++	+/+	/+		30/30	
D291G	Hom	F	Birth	Bilateral		++/++	+/+	+/+	+	28/26	1×
G329V	Het(2^{nd} mut R368H)	?	Birth	Bilateral	0.5/0.6	++/++	+/+			30/30	4×
G329V	Het(2^{nd} mut G61E)	F	Birth	Bilateral	0.5/0.6	++/++	+/+			28/30	
R366fs	Hom	F	<1 year	Left	0.35/0.9	−/−		+/+		17/30	1×
R368C	Hom	F	3 days	Bilateral	0.6/0.5	+/+	+/+	+/+	+		1×
R368C	Het(2^{nd} mut E229K)	F	5 months	Right	0.4/0.6		+/+	+/+		40/12	
M503fs	Het(2^{nd} mut G61E)	F	Birth	Bilateral		++/++	+/+			27/35	Multiple
S506fs	Hom	F	7 months	Bilateral		++/++	+/+	+/+	−	28/28	Multiple

Unknown phenotypic features are left blank.
*Familial/sporadic status of patients: F, familial; S, sporadic; ?, unknown.
†++, very hazy; +, moderately hazy; −, clear.
‡Corneal diameter >12 mm.
§Indicative of rupture of Descemet's membrane.
Hom, homozygous; Cpd Het, compound heterozygous; C/D ratio, cup/disc ratio of optic nerve; En, enucleated; R, right eye; L, left eye.

The insertion g.3318_3319insC (5′NC-487insC) in the 5′ noncoding region of the mRNA (exon 1) was found on all chromosomes of the 50 patients and 10 control individuals sequenced (Table 2). It is not represented in the *CYP1B1* reference gene sequences. However, it is present in GenBank sequence gb/U56438.1, suggesting that this variation is a polymorphism. Because no other sequence variation was found in the chromosomes of the 50 patients sequenced, this exon was not investigated in the remaining patients. Exon 1 of *CYP1B1* has not been extensively investigated in other PCG studies probably because of the absence of mutations in that region. Only one sequence variation in exon 1 (g.3130C>T) has been previously reported as a possible PCG-causing mutation.[25] Q109R and I399V produce conservative amino acid alterations. Furthermore, arginine is found at the position corresponding to amino acid 109 in 15 of 34 aligned cytochrome P450 proteins, and valine is found at the position corresponding to amino acid 399 in one of the aligned proteins (Table 4). I399S was reported as a disease-causing alteration in a French PCG patient.[22] However, serine does not occur at this position in any of the cytochromes sequenced, and this change causes the substitution of a polar amino acid for a nonpolar one. The g.4612C>T variation changes codon AGC to AGT, both of which code serine at position 269. The variation was found in only one control individual, and it is therefore considered a synonymous variation not associated with disease.

Available phenotypic features of patients carrying novel and non-novel mutations associated with PCG are described in Tables 5 and 6, respectively. Among patients carrying homozygous mutations, available data suggest that those with E229K, R368H, and T404fs had the most severe phenotypes. Among compound heterozygous patients, the A202D/G61E, R368C/E229K, and M503fs/G61E genotypes were associated with notably severe phenotypes. The affects of the novel missense mutations on the three-dimensional model of CYP1B1 protein constructed using homology modeling was assessed with the WHATIF structure analysis software program (*http://swift.cmbi.kun.nl/WIWWWI/*). The alterations had no notable affect on H-bonding or surface accessibility parameters (not shown). The templates used for model construction had at least 70% sequence similarity with CYP1B1, and the model constructed had an RMSD value of at least 0.74 Å as compared with the known structure of each of the templates.

CYP1B1 Mutation Frequency and Haplotype Analysis

Putative disease-causing mutations were identified in 139 of the 208 chromosomes investigated, indicating a *CYP1B1* mutation allele frequency of 66.8% among the Iranian PCG patients (Table 1). Nineteen different mutations were found in 72 patients, and no *CYP1B1* mutation was found in 32 of the PCG patients. The large proportion of the Iranian PCG patients carrying homozygous mutations in the *CYP1B1* gene (49 of 72) is indicative of extensive consanguineous marriages in this population.[38] If we assume the *CYP1B1* mutation found in the few patients in whom only one mutation was identified had a role in their disease status, then *CYP1B1* is the cause of disease in 69.2% of Iranian PCG patients.

Among the 47 probands of familial status with *CYP1B1* mutations, nine carried two different mutations (compound heterozygotes), and the rest were homozygous. In one of the homozygous patients, the two identical mutations were carried on different SNP haplotypes, suggesting independent origins. (The intragenic SNP haplotypes identified among the Iranians are presented in Table 7.) Therefore, the disease status of 21% (10 of 47) of the probands of familial cases was not attributable to identity by descent. This signifies a correspondingly high frequency of mutated *CYP1B1* alleles in the Iranian population.

Table 6. Phenotype of PCG Patients with Non-Novel *CYP1B1* Mutations

Mutation	Hom/Cpd Het*	Fam/ Sp†	Age of onset	Effected eye	C/D ratio‡	Corneal opacity§	Edema§	Megalocornea§¶	Haab's striaie§‖	IOP Max‡ (mm Hg)	Surgery (trabeculotomy)
G61E	Hom;15	10/4/1	9:birth; 1:7 days; 2:6 months	11:bilat; 1:R;1:L	0.49	4:++;4:+;6:−	8+;6-	All +	10:+; 6:−	29.1	
G61E	Het(2nd mut R368H);3	1/2/0	2:birth; 1:3 years	3:bilateral	0.48	2:++;2:−	2:+,2:−	4:+;2:−	6:+	23.2	
G61E	Het(2nd mut R390H);4	0/4/0	3:birth; 1:3 years	4:bilateral	0.65			All +	2:+	30.0	
G61E	Het(2nd mut T404fs);1	1/0/0	Birth	Bilateral	0.30	1:++; 1:+	2:+	2:−		22.0	2×
G61E	Het(2nd mut P422R);1	0/1/0	Birth	Bilateral				2:+			1×
G61E	Het(2nd mut R469W);2	2/0/0	1:2 months; 1:4 months	2:bilateral							
G61E	Het(2nd mut not found);1	0/1/0	Birth	Right	0.50		1:+	1:+		27.0	2×
E173K	Hom;2	1/1/0	1:birth;1:8 days	1:bilat;1:R	0.30	3:+	3:+	1:+;3:−		24.0	
E299K	Hom;1	1/0/0		Bilateral		2:+	2:+	4:+	2:+	32.5	
R290fs	Hom;2	2/0/0	1 birth;1:7 months								
R368H	Hom;2	2/0/0	1:birth;1:2 years	1:bilat;1:R	0.87	2:+;2:−	2:+;2:−	3:+	2:−	29.7	
R368H	Het(2nd mut R390H);2	2/0/0	1:birth;1:4 months	2:bilateral	0.7	4:−	4:−	4:+	2:−	24.8	
R368H	Het(2nd mut not found);3	0/3/0	1:birth;1:4 months 1:1 year	3:bilateral	0.58	2:−	2:−	2:−	2:+;4:−	25.0	
R390H	Hom;12	9/2/1	5:birth;1:6 days. 2:3–5 months	12:bilat	0.65	3++;6:+; 1:−	7:+; 3:−	16:+; 8:−	6:+;4:−	29.1	
R390H	Het(2nd mut not found);1	0/1/0	Birth	Bilateral						27.0	Multiple
T404fs	Hom;1	1/0/0	Birth	Bilateral	0.70	1:++;1:−	1:+;1:−	2:+	2:−	31.5	2×
R469W	Hom;7	6/0/1	2:birth; 1:2 months; 1:1 year	7:bilateral	0.60	2:++;1:+;4:−	3:+;3:−	8:+	4:+;4:−	30.0	
No *CYP1B1* mut	32	16/13/3	8:birth; 14:1–6 months; 4:7–12 months	20:bilat; 2:R;4:L	0.59	2:++;13:+;11:−	15:+; 11:−	41:+;5:−	36:+; 18:−	22.9	

*Number after semicolon indicates no. of patients; each heterozygote listed only once.
†Familial/sporadic status of patients: no. familial/no. sporadic/no. unknown status.
‡Average of all eyes.
§Numbers indicate no. of eyes.
¶Corneal diameter >12 mm.
‖Indicative of rupture of Descemet's membrane.

No *CYP1B1* mutation was found in 25% (16 of 63) of familial cases of PCG, whereas the corresponding figure for the sporadic cases was 39% (13 of 33). This difference is consistent with data from other populations wherein *CYP1B1* was less often found to be causative of disease among sporadic as compared with familial cases.[5,39] In Japan, where *CYP1B1* was found to be causative for only 20% of PCG cases, all cases investigated were sporadic.[25]

The g.3987G>A mutation, which produces G61E, was the most frequently mutated *CYP1B1* allele among the Iranian PCG patients, found in 21.6% of the patients' chromosomes examined and in 28.8% of the patients. The next most frequent mutations among the patients' chromosomes were R390H (g.8006G>A), R469W (g.8242C>T), and R368H (g.7940G>A) found in 14.9, 7.7, and 6.3% of patients, respectively. These four common mutations together constituted 50.5% of the PCG

Table 7. *CYP1B1* Haplotype Frequencies of Iranian PCG Patients and Controls

	PCG patients with *CYP1B*1 mutations (n = 72)		PCG patients without *CYP1B1* mutations (n = 32)		Controls (n = 99)	
Haplotype	%	SD	%	SD	%	SD
H1: CCGGTA	62.5	0.10	20.3	0.13	26.0	2.18
H2: TGTCCA	25.3	0.34	29.7	0.03	31.8	2.37
H3: CCGCCA	8.7	0.34	17.2	0.13	24.8	3.05
H4: CCGCCG	3.5	0.0055	32.8	0.13	11.0	2.11
H5: TGTCCG					3.9	1.91
H6: TGTGTA					1.4	1.13
Others (each <1%)					1.1	
Total	100.0		100.0		100.0	

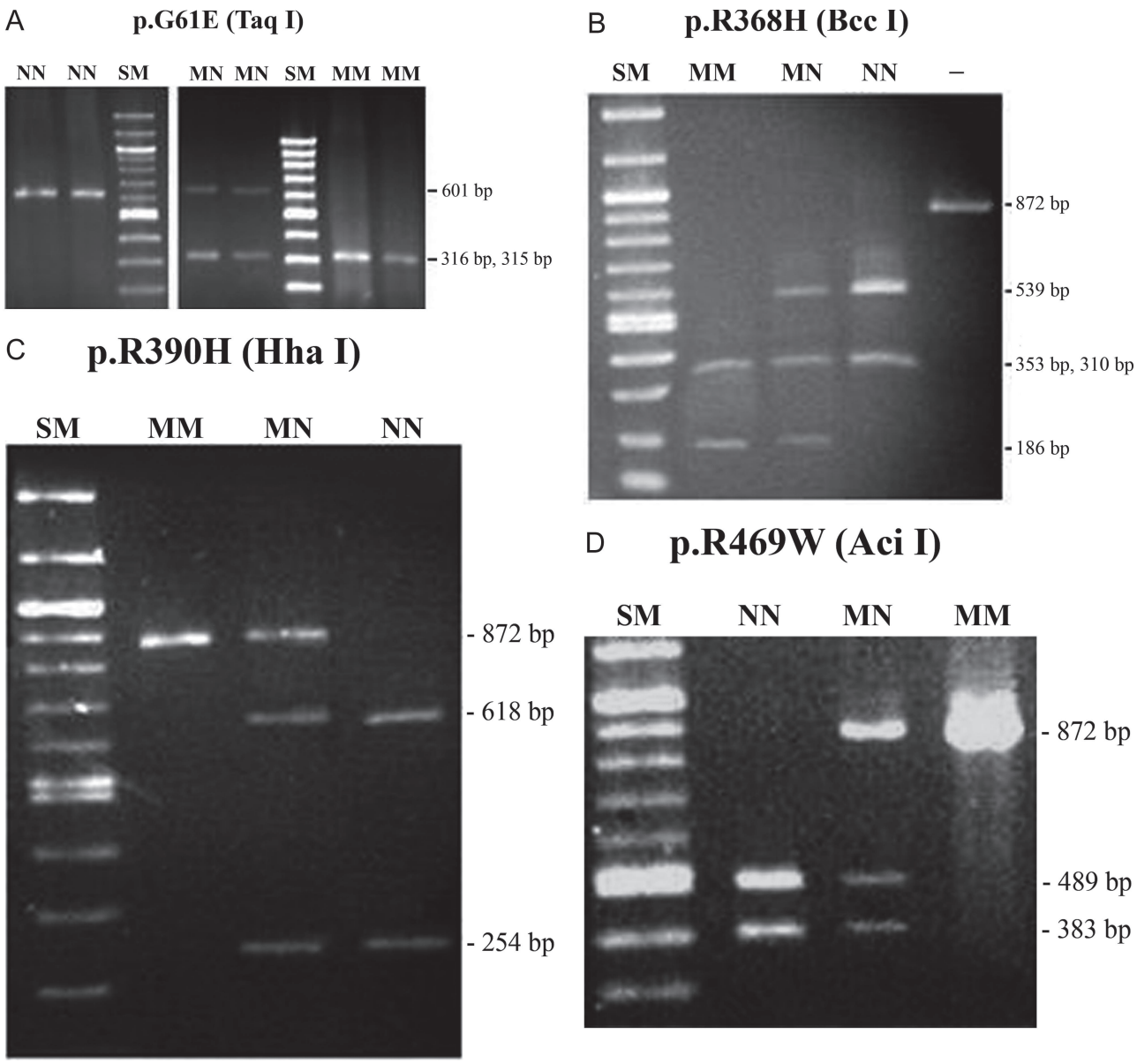

Figure 1. RFLP electrophoresis patterns of common *CYP1B1* mutations of Iranian PCG patients. **A:** g.3987G>A; **B:** g.7940G>A; **C:** g.8006G>A; **D:** g.8242C>T. SM, size markers; NN, homozygous normal; MM, homozygous mutant; MN, heterozygous; −, undigested PCR product. PCR amplicon of exon 2 (primers 2aF and 2aR) was digested for detection of mutation G61E and PCR amplicon of exon 3 for detection of the other three common mutations. *Taq*I digestion of normal and G61E mutated exon 2 amplicons produces 70- and 75-bp fragments that migrate out of the gel. Likewise, *Bcc*I digestion of normal and R368H mutated exon 3 amplicons produces a 23-bp fragment that also migrates out of the gel.

patients' *CYP1B1* alleles and 76.2% of the mutated *CYP1B1* alleles found in the Iranian cohort. They were found in the homozygous or heterozygous state in 66.3% of the patients. The probable contribution of these four mutations to the disease status of the Iranian PCG patients and the clinical implications of this finding directed the design of simple RFLP assays for their detection (Figure 1). The remaining 15 putative disease-causing mutations were each detected in less than 2% of the chromosomes. Six mutations were found in two patients and nine in only one.

Haplotypes based on six intragenic SNPs, four of which have been extensively reported in other studies, were constructed for Iranian PCG patients with and without *CYP1B1* mutations and controls. The six SNPs identified in this population were g.3793T>C, g.3947C>G (R48G), g.4160G>T (A119S), g.8131G>C (V432L), g.8184T>C (D449D), and g.8195A>G (N453S) (Table 2). The genotypes of a majority of the patients carrying *CYP1B1* mutations (54 of 72) were homozygous at all six SNP loci, allowing unambiguous haplotype description. The majority of the heterozygotes were those carrying either the R368H (10 of 18) or R390H (7 of 18) mutations. However, the PCG patients without *CYP1B1* mutations and the control individuals were mostly heterozygous at two or more of these six SNP loci, and the PHASE 2 software was essential for haplotype analysis in these individuals.

Table 8. *CYP1B1* Haplotypes Associated with *CYP1B1* Mutations in PCG Patients

Country	Mutation g.3987G>A (p.G61E)	g.3988delA (p.G61fs)	g.4048C>A (p.Y81X)	g.4322G>A (p.E173K)	g.4410C>A (p.A202D)	g.4490G>A (p.E229K)	g.4611_4619dup9bp (p.N265_R266insSNL)	g 4673_4674insC (p.R290fs)	g.4677A>G (p.D291G)	g.4791G>T (p.G329V)	g.7934delG (p.R366fs)	g.7939C>T (p.R368C)	g.7940G>A (p.R368H)	g.8006G>A (p.R390H)	g.8037_8046dup10bp (p.T404fs)	g.8162C>G (p.P442R)	g.8242C>T (p.R469W)	g.8341delA (p.M503fs)	g.8354_8373del20 (p.S506fs)
Iran (Present study)	1	2	1	2	3	2	3	1	4	1	1	4	1	1,2,3	1	2	1	2	1
Saudi Arabia (Bejjani et al, 2000)	1																1		
Ecuador (Curry et al, 2004)	1																		
India (Chakrabarti et al, 2006)	1					2,6							1,2				1		
Morocco (Belmouden et al, 2002)	1														1				
Brazil (Stoilov et al, 2002)													1		1				

Haplotypes are designated as follows: 1, (C) CGGTA; 2, (T) GTCCA; 3, (C) CGCCA; 4, (C) CGCCG; 6, -_GTGTA-.

Four haplotypes were found among patients with *CYP1B1* mutations and also among patients without *CYP1B1* mutations. These same haplotypes and several additional minor haplotypes were predicted in the controls. The four frequent haplotypes were H1, -CCGGTA-; H2, -TGTCCA-; H3, -CCGCCA-; and H4, -CCGCCG-. The only other haplotypes that reached a frequency of larger than 1% in the control group were H5, -TGTCCG- (3.9%), and H6, -TGTGTA- (1.4%). Estimated frequency distributions of these haplotypes are presented in Table 7. The frequency distributions of the haplotypes are significantly different between PCG patients with and without *CYP1B1* mutations ($P < 0.001$) and between patients with *CYP1B1* mutations and controls ($P < 0.001$). By far the most common (62%) haplotype among patients with *CYP1B1* mutations was H1 (-CCGGTA-). This haplotype was associated with all patients carrying three of the four most common *CYP1B1* mutations (G61E, R368H, and R469W) among the Iranian PCG patients, and these patients made a large contribution to the total frequency of the haplotype (Table 8). H1 was predicted for ~25% of the chromosomes of the Iranian control individuals. It is interesting that the haplotype distribution between PCG patients without *CYP1B1* mutations and controls were also found to be significantly different ($P < 0.01$). The most notable difference relates to haplotype H4 (-CCGCCG-), the frequency of which is approximately three times lower in the control group (11.0 versus 32.8%).

Among the patients, it is apparent that the core haplotypes consist of three blocks with members that consistently co-segregate. The three blocks consist of g.3793T>C, g.3947C>G (R48G), g.4160G>T (A119S); g.8131G>C (V432L), g.8184T>C (D449D); and g.8195A>G (N453S). The same pattern was observed in the predicted genotypes of the vast majority of control individuals (Table 7). The frequency distributions of the blocks between PCG patients with *CYP1B1* mutations and controls are compared in Table 9. The difference in distributions of the first block is not significantly different between the two groups ($P = 0.25$). However, the difference in distributions of the second and third blocks are significantly different ($P < 0.001$ and $P = 0.01$, respectively). The existence of the blocks reflects nucleotide substitution and recombination events during human history, but differences in their frequencies among the groups need to be considered (see the last paragraph of Discussion).

Table 8 presents the haplotype background of *CYP1B1* mutations found among the Iranian PCG patients. For mutations among these that have been previously reported in other populations, Table 8 also shows associated haplotypes in those populations. As recently reported, the mutations are clustered on the background

Table 9. Comparison of Distribution of Blocks within *CYP1B1* Core Haplotypes between PCG Patients with *CYP1B1* Mutations and Control Individuals

	Block 1		Block 2		Block 3	
	TGT	CCG	GT	CC	A	G
PCG patients with *CYP1B1* mutation, $n = 144$*	36	108	90	54	139	5
Controls, $n = 198$*	75	123	51	147	173	25
P	0.25		<0.001		0.01	

Blocks are defined in text.
*Number of alleles.

of the H1 haplotype (-CCGGTA- or _CGGTA-).[26] Most mutations are found on the same haplotype in patients from different countries, suggesting a common origin. For two of the mutations that are associated with multiple haplotypes (R368H and R390H), interchange between the haplotypes of each would require more than one mutation or recombination event. This suggests that these are recurrent mutations without common ancestry. The observation that a high proportion of patients carrying these mutations are compound heterozygotes and are sporadic is consistent with this proposal (Tables 1 and 6). The two haplotypes H1 (-CGGTA-) and H2 (-GTCCA-), associated with R368H and R390H, have been proposed to be ancient human haplotypes.[26] Interchange between two haplotypes associated with E229K could have resulted from a single recombination event between one of these (H2: -TGTCCA-) and the common haplotype H1 (-CCGGTA-). There was no notable difference in the phenotypes of patients carrying the same mutation on different haplotype backgrounds.

Discussion

Considerable sequence heterogeneity was observed in the *CYP1B1* gene among the Iranian PCG patients. High sequence heterogeneity in the *CFTR* gene of cystic fibrosis patients from this population has also been reported.[40] Ten novel mutations were identified, making a notable contribution to the previously reported mutations. This is important because mutations constitute a tool for understanding the biochemical and physiological role of CYP1B1 in the PCG phenotype. It is possible that some mutations were not detected because of the sequencing strategy used. Variations outside the regions of the gene sequenced and large heterozygous deletions would have been missed. Nevertheless, nearly 70% of the Iranian PCG patients carried *CYP1B1* mutations, signifying the clinical importance of this gene. This figure is lower than the corresponding figure for the more homogeneous and inbred populations of Slovakia Roma and Saudi Arabia (~100%). However, it is higher than the corresponding figure for the populations of Brazil and France (~50%) and of the heterogeneous population of India (~40%). The four most common mutations detected among the Iranians were p.G61E, p.R390H, p.R469W, and p.R368H. These together constituted 51% of the Iranian *CYP1B1* alleles studied and 76.2% of the mutated *CYP1B1* alleles observed.

Our results are consistent with a geographic distribution of *CYP1B1* mutations. Two mutated alleles, g.8037_8046dupTCATGCCACC and g.4611_4619delGCAACTTCA (S269_F271del), have been proposed to be ancient on the basis of haplotype analysis and, therefore, are expected to be spread widely; however, only the first was found among the Iranian patients.[41] The second mutation represents deletion of one copy of a 9-bp repeat in the wild-type nucleotide sequence. A further duplication of the repeat was identified as a novel mutation (N265_R266insSNL) among the Iranian patients, suggesting that this may be a mutational hotspot. G61E, the most frequent (~75%) mutant allele among PCG patients of Saudi Arabia, was also the most frequent (21.6%) one among the Iranian patients, although at a significantly lower frequency. The second most common mutation among the Saudi Arabians (R469W) was also one of the common mutations among the Iranian patients.[6] Both mutations occurred on the same haplotype background in the two populations, thus suggesting a common ancestral origin.

R368H has been observed in significant numbers of patients only in India (17%) and is the most common *CYP1B1* mutation in that population.[23,34] It is also one of the most common mutations in Iran, found in 11.5% of the PCG patients. Again, the haplotype background of this mutation in the two populations is the same. R390H, another common mutation found in 19.2% of the PCG patients in Iran, was first identified in a Pakistani patient[42] and subsequently reported in Indian PCG patients[23] and in an early-onset primary open angle glaucoma French patient.[29] E173K, among the more infrequent mutations in the Iranians, was only recently reported as a novel mutation in an Egyptian family,[43] and g.4673_4674insC was previously found in a Turkish pedigree.[14] In contrast, most of the mutations of patients from the American continents and Western Europe were not observed among the Iranians.[41,42] An exception is the E229K mutation, which was reported to be a possible dominant cause of PCG and early-onset primary open angle glaucoma in French patients.[22,29] However, of the two Iranian patients who carried this mutation, one was homozygous and the other also carried a second mutation. The mutations found in patients from Japan and Indonesia seem to be unique to the Far East and have not been reported elsewhere; they were also not found in Iran.[27,36,44,45]

Four core haplotypes defined by six common intragenic SNPs, five of which are coding SNPs, were found among patients with *CYP1B1* mutations and among patients without mutations as well as unaffected controls. The frequency distributions of the haplotypes were significantly different among the groups, with the greatest difference being between the PCG patients with and without *CYP1B1* mutations. The most common haplotype segregating with the mutated alleles was H1 (-CCGGTA-), as has been reported for other populations. The frequency of this haplotype was estimated at 35% in a Saudi Arabian control cohort under the assumption of Hardy-Weinberg equilibrium.[6] The haplotype has a similar frequency among Iranian controls (26%). The haplotype distribution in the Iranian *CYP1B1* mutation group was strikingly similar to that recently reported for the corresponding group of patients from India.[26] The exception was H6 (-_GTGTA-) associated with some E229K alleles among the Indian population and absent among the Iranian patients. The difference in frequency distributions between patients without *CYP1B1* mutations and controls may be attributable to the concentration of the patients without *CYP1B1* mutations within a geographic or ethnic subpopulation not well represented by the controls. Less likely possibilities are that the PCG phenotype in a fraction of these patients is attributable to mutations in parts of the *CYP1B1* gene that were not sequenced,

long-range deletions in *CYP1B1*, or mutations in a gene proximal to *CYP1B1*.

Considering the different mutations individually, haplotype analysis suggests a common origin for most of the mutations. The two mutations R368H and R390H may have occurred more than once during the human evolution. Our data are consistent with the parsimonious scenario of evolution of various *CYP1B1* haplotypes proposed for the human population.[26] It expands on that scenario by extending the length of each of the two proposed ancestral haplotypes by one nucleotide (H1: -CGGTA- to -CCGGTA- and H2: -GTCCA- to -TGTCCA-). It is interesting that descendants of one of the chromosome products (-_GTGTA-) of the proposed recombination event between the ancestral haplotypes is rare both among the PCG patients and the control populations studied. The two proposed ancestral haplotypes have become diluted through human history by mutation and recombination events, and their frequency in the normal population is now comparable with that of two other haplotypes, H3 (-_CGCCA-) and H4 (-_CGCCG-). The finding of most common PCG-causing mutations on the proposed ancestral haplotypes despite their dilution suggests that they are ancient mutations. The more rare mutations often found on haplotypes H3 (-_CGCCA-) and H4 (-_CGCCG-), probably occurred more recently.

Finally, it is evident that the difference in core haplotype distributions between PCG patients with *CYP1B1* mutations and control individuals is almost entirely attributable to differences in distributions of blocks 2 (consisting of V432L and D449D) and 3 (N453S) therein (Table 9). The two alleles at position 432 and the two alleles at position 453 have been reported to code for proteins that differ in enzymatic activity or stability.[18,46] Furthermore, the alleles with higher enzymatic activity or stability (V432, N453) have been found in higher frequencies in patients afflicted with various forms of cancers as compared with normal controls.[19,20,47,48] These same alleles were found to be more frequent in the Iranian cohort of PCG patients with *CYP1B1* mutations as compared with control individuals used in this study (Table 9). For example, the frequencies of the V432 allele in these two groups were 62.5 and 25.8%, respectively. The corresponding numbers for N453 were 96.5 and 87.4%. From an evolutionary perspective, these data are consistent with the proposition that maintenance of PCG-causing mutations, which generally disrupt protein function, may partly serve to compensate for cancer-promoting alterations in the gene sequences.

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