Glaucoma-Associated *CYP1B1* Mutations Share Similar Haplotype Backgrounds in POAG and PACG Phenotypes

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Purpose. To understand the involvement of the *CYP1B1* gene in cases of primary open-angle (POAG) and primary angle-closure (PACG) glaucomas and obtain the haplotype background of these mutations.

Methods. The entire coding region of *CYP1B1* was screened by resequencing in 224 unrelated cases of POAG ($n = 134$) and PACG ($n = 90$) and 200 ethnically matched normal control subjects from Indian populations. Six intragenic single nucleotide polymorphisms (SNPs) in *CYP1B1* (−13T>C, R48G, A119S, V432L, D449D, and N453S) were used to generate haplotype data for the cases and controls and linkage disequilibrium (LD) and haplotype analysis were performed with Haploview software, which uses the EM (expectation–maximization) algorithm.

Results. The frequency of *CYP1B1* mutations was higher among POAG (18.6%; 95% CI, 12.9–26.1) than PACG (11.1%; 95% CI, 6.1–19.3) cases. There was a marked allelic heterogeneity, and the Arg368His was the most prevalent mutation across both the phenotypes. The spectrum of *CYP1B1* mutations was largely similar across different POAG populations. Haplotypes generated with intragenic SNPs indicated the C-C-G-G-T-A to be a risk haplotype associated with *CYP1B1* mutations in POAG ($P = 0.006$) and PACG ($P = 0.043$), similar to that observed in cases of primary congenital glaucoma worldwide.

Conclusions. The results demonstrate an involvement of *CYP1B1* in a proportion of POAG and PACG cases that should be explored further. The similar haplotype background of these mutations is indicative of their common origin across multiple glaucoma phenotypes. (*Invest Ophthalmol Vis Sci.* 2007;48:5439–5444) DOI:10.1167/iovs.07-0629

Glaucoma comprises a group of clinically and genetically heterogeneous optic neuropathies characterized by a progressive loss of vision and is the second leading cause of irreversible blindness worldwide.[1,2] Based on gonioscopic findings, primary glaucomas are classified as primary open-angle glaucoma (POAG; OMIM 137750; Online Mendelian Inheritance in Man; http://www.ncbi.nlm.nih.gov/Omim/ provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD) and primary angle-closure glaucoma (PACG). Both have characteristic optic nerve head changes, degeneration of retinal ganglion cells, and visual field loss, but PACG also has a closed angle or peripheral anterior synechia (PAS) on gonioscopy.[3] Both of these conditions may be associated with elevated intraocular pressure (IOP) due to the obstruction of outflow.[4] POAG affects 33 million worldwide and is more common in the West,[2,5,6] whereas PACG is relatively more common among Asian populations.[7,8]

Genetic heterogeneity is well documented in POAG, and 11 chromosomal loci (*GLC1A-GLC1K*) have been mapped by linkage analysis.[9] Of these, only three genes harboring *GLC1A* (Myocilin; *MYOC*; OMIM 601652),[10] *GLC1E* (Optineurin; *OPTN*; OMIM 602432),[11] and *GLC1G* (*WDR36*; OMIM 609669)[12] have been characterized. In addition, approximately 15 candidate genes have been identified by association studies that require a thorough replication in different populations.[9] Glaucoma being a complex disease would be attributed to multiple gene variants with various magnitudes of effect.[13]

Although the human cytochrome P450 gene *CYP1B1* (OMIM 601771) has been implicated in primary congenital glaucoma (PCG; OMIM 231300) worldwide,[14–16] it has been relatively less explored in POAG and not at all in PACG. An initial study implicated the involvement of *CYP1B1* with *MYOC* through a digenic mechanism in a family with juvenile-onset open angle glaucoma (JOAG) and suggested that *CYP1B1* is a modifier of *MYOC* expression. It was also observed that affected subjects harboring a mutant *CYP1B1* allele in this family had an earlier age at onset than those with only a mutant *MYOC* allele.[17] These findings led to the screening of *CYP1B1* as a candidate gene among the patients with POAG and largely among those with JOAG. The frequency of *CYP1B1* mutations varied in patients from Canada (5.0%),[17] France (4.6%),[18] Spain (10.9%),[19] Eastern India (4.5%),[20] and Southern India (10.8%).[21] The differences in mutation frequency could be partly explained by the definition of disease used in these studies. The Canadian patients had JOAG[17] whereas the French patients had POAG, but elevated IOP was not an inclusion criterion,[18] similar to studies from Eastern[20] and Southern[21] India. The results from these studies indicate a minor involvement of *CYP1B1* among JOAG and late-onset POAG cases and suggest a possible role of this gene in glaucoma pathogenesis.

We have reported the extent of *CYP1B1* mutations along with their structural properties in PCG.[22,23] We have also demonstrated a global clustering of these mutations on specific haplotype backgrounds, irrespective of geographic location, that could be useful in predictive testing.[16] Herein, we report an extensive screening of the *CYP1B1* gene in a cohort of patients with POAG or PACG from India, to determine its mutation spectrum and understand the haplotype backgrounds of these mutations.

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METHODS

Clinical Details of the Subjects

The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board. The cohort comprised unrelated, consecutive patients with JOAG ($n = 30$), POAG ($n = 104$), PACG ($n = 90$), and 200 normal control subjects, who were seen at the L. V. Prasad Eye Institute (Hyderabad, India) between January 2002 and March 2007. The diagnoses of POAG and PACG were independently confirmed by two surgeons based on the following inclusion and exclusion criteria.

POAG (Including JOAG)

The diagnosis of POAG was based on open angles on gonioscopy, an IOP >21 mm Hg, and characteristic optic disc changes and corresponding visual field defects in patients >35 years of age. Visual field defects were considered to be glaucomatous if they were consistent with optic disc damage and met at least two of the criteria laid out by Anderson and Patella.[24] The presence of a visual field defect required confirmation by a repeatable field performed within 2 weeks of the first reliable visual field result showing the defect. The field defects were further classified as mild, moderate, or severe.[25] Such findings in patients between 5 and 35 years of age were labeled as JOAG. As the presence of visual field defects was one of the inclusion criteria, only patients older than 10 years were included in the study.

Primary Angle-Closure Glaucoma

PACG was defined as the presence of optic disc and visual field changes characteristic of glaucoma, along with appositional or synechial primary angle-closure (PAC) in patients older than 18 years. The visual field defects were as defined in POAG. PAC (appositional) was defined as increased IOP (>21 mm Hg) associated with nonvisibility of the filtering trabecular meshwork for more than 180°, in the absence of PAS, disc damage, or field changes. PAC (synechial) was defined as the presence of PAS with nonvisibility of the filtering trabecular meshwork for more than 180°, with or without increased IOP (>21 mm Hg), without disc damage or demonstrable field defects. The presence of even a single PAS in an angle with more than 180° of nonvisibility of trabecular meshwork was considered diagnostic of PAC. Other causes of synechiae were excluded.

Ocular hypertension, normal-tension glaucoma, lens-induced glaucoma, neovascular and pseudoexfoliation glaucoma, and secondary open-angle glaucoma were excluded. Other ocular diseases that can lead to secondary glaucoma were also excluded.

Normal adult individuals without any signs or symptoms of glaucoma and other systemic diseases served as control subjects. Their visual acuity ranged from 20/20 to 20/40, and their IOP was <21 mm Hg. Clinical examination on stereo biomicroscopy did not reveal any changes in the optic disc suggestive of glaucoma. The patients and controls were matched with respect to their ethnicity and geographical region of habitat.

Molecular Analysis

Peripheral blood samples (5–10 mL) were collected from each subject by venipuncture, with prior informed consent. DNA was extracted by standard protocols[26] and the entire coding region of *CYP1B1* was amplified using appropriate oligonucleotide primers and PCR protocols, as published earlier.[27] The amplicons were purified (SigmaSpin columns; Sigma-Aldrich, St. Louis, MO) and bidirectionally sequenced using dye termination chemistry (BigDye on a 3100 DNA Analyzer; Applied Biosystems, Inc. [ABI], Foster City, CA), according to the manufacturer's protocol. Sequencing analysis software was used to read the individual sequences. Six mutations (G61E, Y81N, Q144R, P193L, E229K, and R368H) were further confirmed by restriction digestion of the amplicon with appropriate restriction enzymes as published earlier,[18,22,27] whereas the remaining five mutations were verified by resequencing. Multiple sequence alignment of the human CYP1B1 protein was performed along with other CYP1 protein across different families, to check for the conservation of the residues. The SIFT (sorting tolerant from intolerant) homology tool (http://blocks.fhcrc.org/sift/SIFT.html/ provided in the public domain by the Fred Hutchinson Cancer Research Center, Seattle, WA) was used to assess the effect of the substituted amino acid on the CYP1B1 protein, and a threshold score of less than 0.05 was considered to be deleterious to the protein.[28]

Statistical Analysis

The maximum-likelihood estimates of allele frequencies, Hardy-Weinberg equilibrium, and haplotype frequencies were estimated from the genotype data at six single-nucleotide polymorphism (SNP) loci using Haploview software, which uses the EM (expectation–maximization) algorithm.[29] Pair-wise linkage disequilibrium (LD) between the individual SNPs was calculated using the LD-plot function of this software. The odds ratios were calculated, to assess the risk of the individual genotypes at all six SNP loci. Clinical parameters, such as IOP at presentation, cup-to-disc ratio, and visual field defects for the worst eye were considered when correlating the genotype with the phenotype. All calculations were performed with commercial software (SPSS ver. 14; SPSS, Chicago, IL).

RESULTS

Mutation Screening of *CYP1B1* in POAG and PACG

The study cohort conformed to Hardy-Weinberg equilibrium. A total of 11 *CYP1B1* mutations were observed, of which 4 (Q144R, W434R, F445C, and g.8148-8152del5bp) were novel. The overall spectrum of *CYP1B1* mutations observed in POAG and PACG is demonstrated in Figure 1 (the electropherograms

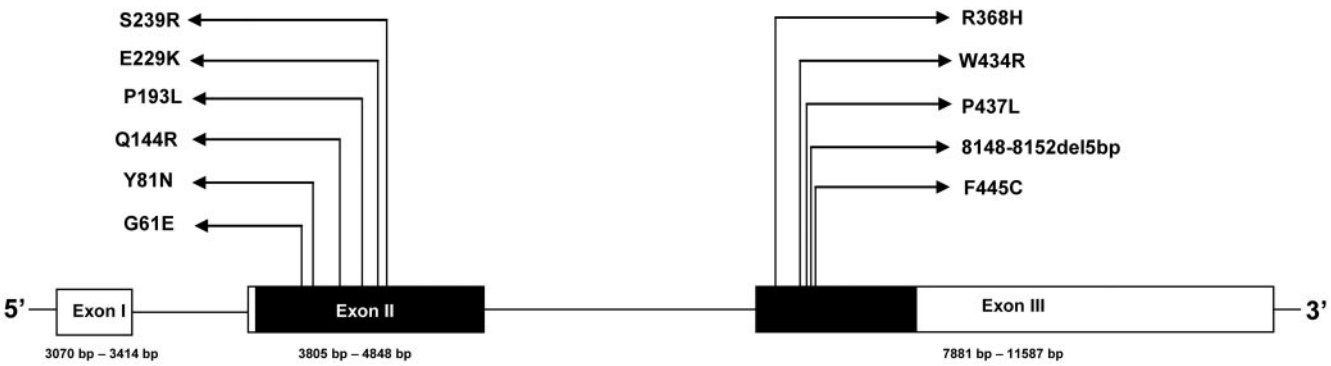

FIGURE 1. Schematic representation of the *CYP1B1* mutations observed in POAG and PACG. *Arrows*: location of the mutations within the *CYP1B1* gene. *Shaded regions*: coding regions in exon II and III of *CYP1B1* gene.

of all 11 mutations are provided in Supplementary Fig. S1, online at http://www.iovs.org/cgi/content/full/48/12/5439/DC1). The frequency of mutations was higher in POAG (18.6%; 25/134) than in PACG (11.1%; 10/90). Arg368His was the most prevalent mutation across both the phenotypes, similar to earlier studies of PCG from India.[16,22] Further details are provided in Table 1. Allelic heterogeneity was relatively more in POAG than in PACG. SIFT scores indicated a deleterious effect for all the mutations except E229K.

The cosegregation of the heterozygous mutant allele was observed in only three families for the three mutations (G61E, Q144R, and P193L). DNA samples were unavailable from the relatives of the probands in the remaining cases harboring *CYP1B1* mutations. Except for the Q144 residue, multiple sequence alignment indicated a strong conservation of the wild-type residues for all amino acids across multiple CYP1 families (Supplementary Fig. S2, http://www.iovs.org/cgi/content/full/48/12/5439/DC1).

Homozygosity of the mutant allele was noted in a JOAG case with G61E and in a POAG case with P193L mutations. There was only a single JOAG case with a compound heterozygous mutation (G61E and R368H). All other mutations were observed in the heterozygous state in JOAG (5/7), POAG (17/18), and all PACG.

The *CYP1B1* mutation frequencies were different across all the studies performed on POAG in Indian populations.[20,21] Of interest, the investigators in the study from Southern India found a carrier rate of 6.4% and 0.7% for the E229K and the R368H mutations, respectively, in their control populations[21] that was not observed in the cohorts from Eastern India[20] or in the present study.

Table 2 provides a comparison of JOAG, POAG, and PACG cases. As is evident from the table, JOAG cases had a higher prevalence of *CYP1B1* mutations than did POAG cases. There was no significant difference in age at onset among JOAG cases with (20.1 ± 8.78 years) and without (20.9 ± 8.31 years) *CYP1B1* mutations ($P = 0.781$). JOAG cases had a significantly higher mean IOP at presentation than did POAG cases, with and without *CYP1B1* mutations ($P < 0.001$). The mean IOPs were similar among the JOAG and PACG cases with and without mutations. *CYP1B1* mutations did not seem to be associated with disc changes ($P = 0.192$) and severe visual field defects ($P = 0.417$) in any of these phenotypes.

Linkage Disequilibrium and Haplotype Analysis at the *CYP1B1* Locus

Six intragenic SNPs were typed at the *CYP1B1* locus, to generate haplotypes among the cases and controls. Pair-wise LD analysis indicated strong LD ($D' = 1$) at two clusters, between three SNPs (−13T>C, R48G, and A119S) and between the two SNPs V432L and D449D (data not shown). The measure of LD between the other SNPs was similar to that in an earlier study.[35]

Four different haplotypes (with frequency >5%) were generated with these six SNPs in cases and controls. There were no significant differences in the haplotype frequencies when all POAG and PACG cases were compared with the controls (Tables 3, 4). Reanalysis of the cases with respect to their mutation status indicated a significantly higher frequency of the C-C-G-G-T-A haplotype in both POAG ($P = 0.006$) and PACG ($P = 0.043$) cases with *CYP1B1* mutations (*CYP1B1*$^+$) than controls. However, there was no observable difference in frequencies of the other haplotypes among cases and controls. The significantly higher frequency of the C-C-G-G-T-A haplotype in POAG ($P = 0.001$) and PACG ($P = 0.020$) cases with *CYP1B1* mutations was consistent, even when compared with cases without (*CYP1B1*$^-$) mutations (data not shown).

TABLE 1. Details of *CYP1B1* Mutations Observed in Cases of POAG and PACG in the Present Study and Other Populations

Nucleotide Change	Location	Amino Acid Change	Restriction Site Generated†	SIFT Score	Number of Patients with the Mutation			Controls with the Change	Mutation Previously Observed in Other Populations	
					JOAG	POAG	PACG		JOAG/POAG	PCG
g.3987G>A	Exon II	G61E	*TaqI* (+)	0.00	2/30	—	—	0/200	Spain[19]	India,[22] Turkey,[30] Ecuador,[31] Saudi Arabia,[30,32] Morocco,[33] Kuwait[34]
g.4046T>A	Exon II	Y81N	*HaeII* (−)	0.00	1/30	—	—	0/200	Spain,[19] France[18]	Germany[35]
g.4236A>C	Exon II	Q144R*	*MspA1I* (−)	0.01	1/30	1/104	1/90	0/200	—	—
g.4381C>T	Exon II	P193L	*Eco81I* (+)	0.01	—	3/104	—	0/200	India[21]	India[22]
g.4491G>A	Exon II	E229K	*Eam1104I* (−)	0.10	1/30	4/104	4/90	0/200	Spain,[19] France,[18] India[20,21]	India,[22] France[36]
g.4520A>C	Exon II	S239R	—	0.00	—	1/104	—	0/200	—	India[22]
g.7940G>A	Exon III	R368H	*TaaI* (−)	0.00	1/30	6/104	5/90	0/200	India[20,21]	India,[22] Brazil,[37] Saudi Arabia,[30,32] Kuwait[34]
g.8137T>C	Exon III	W434R*	—	0.00	—	1/104	—	0/200	—	—
g.8147C>T	Exon III	P437L	—	0.00	—	1/104	—	0/200	—	India,[22] Brazil,[37] Turkey[30]
g.8171T>G	Exon III	F445C*	—	0.03	—	1/104	—	0/200	—	—
g.8148-8152del5bp	Exon III	Frame shift*	—		1/30	—	—	0/200	—	—
Total					7/30	18/104	10/90	0/200		

* Novel mutation.

† Restriction enzymes that were used to confirm the different mutations: (+) gain and (−) loss of restriction sites.

TABLE 2. Distribution of Mean Ages at Onset and IOPs at Presentation among JOAG, POAG, and PACG Groups

Parameters	JOAG Cases with *CYP1B1* Mutations (n = 7)	JOAG Cases without *CYP1B1* Mutations (n = 23)	POAG Cases with *CYP1B1* Mutations (n = 18)	POAG Cases without *CYP1B1* Mutations (n = 86)	PACG Cases with *CYP1B1* Mutations (n = 10)	PACG Cases without *CYP1B1* Mutations (n = 80)
% Frequency of Cases (95% CI)	23.3 (11.8–40.9)	76.7 (59.1–88.2)	17.3 (11.2–25.7)	82.7 (74.3–88.7)	11.1 (6.1–19.2)	88.9 (80.7–93.8)
Age at Onset (mean years ± SD)	20.1 ± 8.78	20.9 ± 8.31	51.3 ± 12.22	54.1 ± 10.56	57.4 ± 12.43	54.4 ± 11.18
IOP at Presentation (mean mm Hg ± SD)	37.3 ± 8.43	34.1 ± 8.33	24.8 ± 3.04	27.1 ± 6.26	32.6 ± 16.73	32.9 ± 10.11

TABLE 3. Distribution of Estimated *CYP1B1* Haplotype Frequencies among POAG Cases and Controls

Haplotypes	POAG (All Cases)	Controls	P	POAG *CYP1B1*(+)†	Controls	P	POAG *CYP1B1*(−)‡	Controls	P
T-G-T-C-C-A	36.7%	44.8%	0.079	40.1%	44.7%	0.557	36.0%	44.7%	0.072
C-C-G-G-T-A	21.2%	20.1%	0.809	38.4%	20.1%	0.006*	17.9%	20.1%	0.535
C-C-G-C-C-A	22.2%	18.2%	0.287	9.9%	18.3%	0.143	24.3%	18.3%	0.132
C-C-G-C-C-G	17.4%	13.5%	0.252	7.5%	13.3%	0.253	20.6%	13.5%	0.058

* Significant.
† Cases harboring *CYP1B1* mutation.
‡ Cases without *CYP1B1* mutations.

TABLE 4. Distribution of Estimated *CYP1B1* Haplotype Frequencies among PACG Cases and Controls

Haplotypes	PACG (All Cases)	Controls	P	PACG *CYP1B1*(+)†	Controls	P	PACG *CYP1B1*(−)‡	Controls	P
T-G-T-C-C-A	36.8%	44.8%	0.099	44.9%	44.6%	0.978	37.3%	44.8%	0.130
C-C-G-G-T-A	20.8%	20.1%	0.861	39.9%	20.1%	0.043*	18.3%	20.1%	0.660
C-C-G-C-C-A	21.3%	18.5%	0.463	10.1%	18.4%	0.352	22.1%	18.5%	0.372
C-C-G-C-C-G	14.0%	13.3%	0.831	5.0%	13.3%	0.284	14.6%	13.3%	0.698

* Significant.
† Cases harboring *CYP1B1* mutation.
‡ Cases without *CYP1B1* mutations.

DISCUSSION

The present study provides a mutation spectrum of the *CYP1B1* gene in POAG and PACG. The involvement of *CYP1B1* highlights its role as a potential candidate in disease pathogenesis that should be explored further. We observed a higher proportion of mutations in POAG in the present cohort than in other populations (Table 5). The spectrum of mutations observed in the present cohort was largely similar to that in the POAG populations in France, Spain, and India.[18–21] Except for the four novel mutations observed in this study, all other variants were observed earlier in patients with PCG in India and other countries (Table 1).

To the best of our knowledge, this is also the first study to report the involvement of *CYP1B1* in PACG. Although there are differences in the mutation frequencies of *CYP1B1* across JOAG, POAG, and PACG, the 95% CI of these frequencies overlap (Table 2). It would also be interesting to investigate the role of *CYP1B1* mutations in PAC, where there is no damage to the disc and visual fields, as opposed to PACG, where disc and fields are affected. Although that would be the subject for further study, we have screened 16 PAC cases, and none of them had *CYP1B1* mutations (95% CI, 0–17.57). Although this was not significantly different from PACG cases with *CYP1B1* mutations (as 95% CI overlaps; Table 2), the sample size was too small to draw any conclusion. Further studies on a larger sample are needed to determine *CYP1B1* involvement in PAC.

Although we observed a higher mutation frequency of *CYP1B1* in POAG than in other populations, our results are not very different from those in a Spanish population,[19] when we look at the confidence intervals in these two studies (Table 5). The frequency differs, however, from those in French and other Indian populations. These differences may be partially attributable to the definitions of POAG used in these studies.[18,20,21] In contrast to the French and other Indian studies, we used raised IOP (>21 mm Hg) in the definition of POAG and PACG, as it was our inclusion criterion. It is well known that *CYP1B1* is a major candidate gene in PCG that is associated with increased IOP.[14–16] Hence, this could partially explain the higher frequency of *CYP1B1* mutations in our patient cohort. The report on the Spanish patients with POAG[19] also included increased IOP (>21 mm Hg) as a major inclusion criterion, and, as just noted, their mutation rates are not very different from ours (Table 5).

It is interesting to note that the prevalent mutation was different across all previously reported POAG populations (Table 5). Also, the frequency of heterozygous mutations was similar across these studies. Although the R368H mutation was common in patients in both the Indian and Canadian studies, it

TABLE 5. Characteristics of *CYP1B1* Mutations across Different POAG Populations

Populations (cases)	Median Age at Onset in Years (Range)	% Frequency of *CYP1B1* Mutation (95% CI)	% Frequency of Heterozygous *CYP1B1* Mutation (95% CI)	Prevalent *CYP1B1* Mutation
Canada (n = 60)[17]	23.6 (8–36)	5.0 (1.7–13.7)	66.6 (20.8–93.8)	R368H
France (n = 236)[18]	40 (13–52)	4.6 (2.6–8.2)	90.9 (62.3–98.4)	A443G
Spain (n = 82)[19]	59.9 (48–77)	11.0 (5.9–19.6)	100.0 (70.1–100.0)	Y81N
Eastern India (n = 200)[20]	NA*	4.5 (2.4–8.3)	88.9 (56.5–98.1)	S515L
Southern India (n = 251)[21]	NA*	10.7 (7.5–15.2)	92.5 (76.6–97.9)	E229K
Present study; India (n = 134)	46 (10–80)	18.6 (12.9–26.1)	88.0 (80.2–93.0)	R368H

* Not available.

was noted in only 2 of the 60 patients with JOAG in the Canadian report.[17] One of the Canadian patients with the R368H mutation had an East Indian/Guyanese ancestry,[17] but we were not able to determine whether this patient shared a common haplotype background with the Indian patient due to unavailability of data.

The median age at onset of the patients with POAG in the present cohort was similar to that of the French sample,[18] but was significantly lower than that of the Spanish[19] patients with POAG. The median age of the Canadian patients was significantly lower, as no cases older than 40 years were enrolled.[17] Another study on patients with POAG from Eastern India[20] reported a mutation frequency (4.5%) similar to that of the French population but a higher mean age (52.43 ± 19.33 years) than that of our cohort. Of interest, the prevalent mutation in the Eastern Indian (S515L)[20] and Southern Indian (E229K)[21] cohort was also different from that in the present study (R368H).

Another interesting observation was the presence of *CYP1B1* mutations on specific haplotypes that was earlier observed in PCG.[16] We noted that C-C-G-G-T-A was the risk haplotype in cases of POAG and PACG with *CYP1B1* mutations. These results were consistent (even after reanalyzing the data set) based on a five-locus haplotype (i.e., C-G-G-T-A), similar to previous studies in different PCG populations worldwide.[16,32,33,35,37] On the other hand, the G-T-C-C-A haplotype that was largely associated with the unaffected controls and PCG cases without *CYP1B1* mutations[16] was similar in frequency in the POAG and PACG cases with *CYP1B1* mutations and the controls (Tables 3, 4). In tune with our previous study on PCG,[16] most of the mutations observed in the POAG and PACG clustered on the C-G-G-T-A haplotype. The R368H mutation, which was the prevalent mutation in POAG and PACG in the present study, similar to PCG in India,[16,22] was found on the background of the C-G-G-T-A haplotype across all these phenotypes. Of interest, this mutation was also found on the same haplotype in Saudi Arabian[32] and Brazilian[37] PCG patients. The G61E mutation in POAG was also found on the C-G-G-T-A haplotype, similar to that observed among the PCG patients from Ecuador,[31] Saudi Arabia,[32] and Morocco.[33] The E229K mutation that was observed on the G-T-C-C-A haplotype among patients with PCG in India[16] and Germany[35] was also seen to harbor the same mutation in POAG and PACG cases. Another striking similarity was the presence of the Y81N mutation in a case of POAG on the G-T-C-C-A haplotype. This mutation was also found on the same haplotype among German patients with PCG.[35]

Similar to our earlier hypothesis on the evolution of *CYP1B1* mutations, we confirm that there is a strong clustering of these mutations on specific haplotype backgrounds, irrespective of geographical location.[16] A larger proportion of mutations were seen on the C-G-G-T-A haplotype and a smaller proportion on the G-T-C-C-A haplotype, further confirming the former to be an ancient haplotype and the latter to be a recent haplotype.[16] A formal haplotype comparison among other POAG cases with *CYP1B1* mutations was not possible due to the unavailability of data from other populations. Based on the present analysis we speculate that the presence of specific *CYP1B1* mutations on specific haplotype backgrounds in PCG worldwide and in patients with POAG and PACG in the present cohort is indicative of common founders. The mutations on these haplotypes would have migrated across different geographical regions due to population movements as reported in PCG in our previous study.[16] Thus, glaucoma-associated *CYP1B1* mutations share a similar haplotype background across POAG, PACG, and PCG.

The role of CYP1B1, particularly in retinoic acid synthesis is pivotal during embryonic development. Recent studies on chick embryogenesis have demonstrated its importance in the dorsoventral patterning of the neural tube that is consistent with its endogenous expression.[38] Several in vitro and in vivo studies in lower organisms have demonstrated the sites of expression of CYP1B1 at different stages of development in the anterior retina and anterior segment of the eye.[38–41] Although these studies have provided convincing evidence of the possible role of CYP1B1, its actual molecular mechanism leading to glaucoma in humans has to be deciphered. Although the functions of *CYP1B1* mutations leading to POAG and PACG remain to be characterized, it is nevertheless an important candidate gene that should be screened in patients with glaucoma worldwide, to establish its involvement in the disease's pathogenesis.

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