The Transcription Factor Gene *FOXC1* Exhibits a Limited Role in Primary Congenital Glaucoma

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Purpose. Primary congenital glaucoma (PCG) is an autosomal recessive disorder that has been linked to *CYP1B1* mutations. This study was conducted to explore the role of *FOXC1*, which is involved in anterior segment dysgenesis, in PCG.

Methods. An earlier screening for *CYP1B1* in a clinically well-characterized PCG cohort ($n = 301$) revealed cases that were either homozygous ($n = 73$), compound heterozygous ($n = 18$), or heterozygous ($n = 41$) for the mutant allele, whereas the remaining ($n = 169$) did not harbor any mutation. Hence, *FOXC1* was screened in 210 PCG cases who were either heterozygous ($n = 41$) or did not harbor any *CYP1B1* mutation ($n = 169$), along with ethnically matched normal control subjects ($n = 157$) by resequencing the entire coding region.

Results. Two heterozygous missense (H128R and C135Y) and three frame shift mutations (g.1086delC, g.1155del9bp, and g.1947dup25bp) were observed in *FOXC1* in 5 (2.38%) of 210 cases. The missense mutations had a de novo origin in two sporadic cases, whereas the *FOXC1* deletions were seen in two cases that were also heterozygous for the *CYP1B1* allele (R368H). The parents of the proband with g.1086delC were heterozygous for either the *FOXC1* or *CYP1B1* alleles. The unaffected mother of the proband with the g.1155del9bp was heterozygous for both the *FOXC1* and *CYP1B1* alleles; the father harbored only the *FOXC1* allele. Familial segregation of the g.1947dup25bp could not be performed because of the unavailability of DNA from one parent. Except for the g.1155del9bp (0.95% normal chromosomes), all the other variations were absent in the control subjects.

Conclusions. The present study indicates a limited role of *FOXC1* in PCG pathogenesis. (*Invest Ophthalmol Vis Sci.* 2009;50:75–83) DOI:10.1167/iovs.08-2253

Primary congenital glaucoma (PCG; OMIM 231300) is an autosomal recessive disorder of the eye, caused by a developmental defect in the trabecular meshwork and anterior chamber angle. This condition leads to elevated intraocular pressure (IOP) due to the obstruction of aqueous outflow and resultant optic nerve damage, which if untreated, results in irreversible blindness.[1,2] The signs and symptoms of PCG are usually observed at birth or in early infancy.[3] The prevalence of PCG is very high among the inbred populations such as Slovakian Gypsy (1:1250),[4] Saudi Arabians (1:2500)[5] and Indian inhabitants of Andhra Pradesh (1:3300)[6]; it is relatively lower in Western populations (1:20,000–1:10,000).[3]

Genetic heterogeneity is the hallmark of PCG and three chromosomal loci on 2p21 (*GLC3A*; OMIM 231300 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),[7] 1p36 (*GLC3B*; OMIM 600975),[8] and 14q24.3 (*GLC3C*; Stoilov IR, et al. *IOVS* 2002;43:ARVO E-Abstract 3015) have been mapped by linkage analysis, of which only the *GLC3A* locus harboring the human cytochrome P450 gene *CYP1B1* (OMIM 601771) has been characterized.[9] *CYP1B1* exhibits a high degree of allelic heterogeneity, and more than 70 different mutations causal to PCG have been identified.[10] The proportion of PCG cases attributable to *CYP1B1* mutations vary widely across populations and are highest among the inbred Slovakian Gypsy[4] and Saudi Arabian[5] populations, which exhibit allelic homogeneity. The frequency of *CYP1B1* mutations in other populations varies widely from ~14% to 70% worldwide, and the common mutations are strongly clustered on specific haplotype backgrounds, irrespective of their geographic locations, indicating strong founder effects.[11,12]

Earlier, we showed that a small proportion of PCG cases that do not harbor *CYP1B1* mutations exhibit a heterozygous mutation in the myocilin gene (*MYOC*; OMIM 601652), which causes primary-open angle glaucoma.[13] Digenic inheritance of the mutant *MYOC* and *CYP1B1* alleles has also been demonstrated in juvenile-onset POAG and *CYP1B1* has been suggested to be a modifier of *MYOC* expression.[14]

The *F*orkhead B*ox C1* gene (*FOXC1*; OMIM 601090) is a member of the winged helix/forkhead family of transcription factors and has a highly conserved 110-amino-acid DNA-binding domain, known as the forkhead domain (FHD). It is located on 6p25 and has a single exon that codes for a protein of 553 amino acids.[15] The FOXC1 protein is expressed in various ocular and nonocular tissues.[16–21] It is found in the periocular mesenchyme cells that give rise to ocular drainage structures such as the iris, cornea, and TM.[22] Both the *FOXC1* null ($Foxc1^{-/-}$) and the heterozygous ($Foxc1^{+/-}$) mice were found to have anterior segment abnormalities similar to those in humans with anterior segment dysgenesis (ASD) and congenital glaucoma, such as small or absence of Schlemm's canal, aberrantly developed TM, iris hypoplasia, severely eccentric pupils, and displaced Schwalbe's line.[23]

In addition, *FOXC1* mutations have been implicated in ASD, such as iridogoniodysgenesis, Axenfeld-Rieger syndrome (ARS), and Peter's anomaly that progress to glaucoma in 50% to 75% of affected cases.[17,18,24,25] Some of these ASD cases are associated with congenital or early-onset glaucomas, whereas some have glaucoma secondary to anterior segment anomalies.[15,18,25] These findings indicate a potential role of the transcription factor *FOXC1* in the development of ocular tissues

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Table 1. Primer Sequences Used to Amplify the *FOXC1* Coding Region

Serial No.	Primer Sequence (5′–3′)	Amplicon Size (bp)
FOXC1-1F	CCCGGACTCGGACTCGGC	427
FOXC1-1R	AAGCGGTCCATGATGAACTGG	
FOXC1-2F	CGGCATCTACCAGTTCATCAT	240
FOXC1-2R	TCTCCTCCTTGTCCTTCACC	
FOXC1-3F	GAGAACGGCAGCTTCCTG	298
FOXC1-3R	TGTCGGGGCTCTCGATCTT	
FOXC1-4F	AGATCGAGAGCCCCGACA	184
FOXC1-4R	GCAGCGACGTCATGATGTTG	
FOXC1-5F	CAACATCATGACGTCGCTG	262
FOXC1-5R	TTGCAGGTTGCAGTGGTAGGT	
FOXC1-6F	GGCCAGAGCTCCCTCTACA	245
FOXC1-6R	GTGACCGGAGGCAGAGAGTA	
FOXC1-7F	TCACCAGCAGCAGCTCGT	231
FOXC1-7R	ACTCGAACATCTCCCGCA	
FOXC1-8F	TCACAGAGGATCGGCTTGAA	165
FOXC1-8R	CTGCTTTGGGGTTCGATTTA	

including the drainage structures. But, to the best of our knowledge, none of these studies involved classic PCG cases that are not associated with any anterior segment anomalies. Moreover, since neither *CYP1B1* nor *MYOC* could explain the overall genetic contribution to PCG in earlier studies,[11–13] we sought to screen *FOXC1* as a candidate gene in a PCG cohort from India that were either heterozygous for a mutant *CYP1B1* allele or did not harbor any mutations.

Methods

Selection of Cases

The study protocol adhered to the guidelines of the Declaration of Helsinki and was approved by the Institutional Review Board. Our ongoing screening of *CYP1B1* in a large clinically well-characterized and unrelated PCG cohort ($n = 301$) revealed mutations in 132 (43.8%) cases. The group comprised cases that were either homozygous ($n = 73$), compound heterozygous ($n = 18$), or heterozygous ($n = 41$) for the mutant allele. The remaining cases ($n = 169$) did not harbor any *CYP1B1* mutation. Thus, we chose to screen *FOXC1* in 210 PCG cases that had either no ($n = 169$) or one ($n = 41$) copy of the mutant *CYP1B1* allele. Ethnically matched and unrelated normal volunteers without any sign or symptoms of glaucoma or other ocular or systemic diseases were enrolled as control subjects ($n = 157$).[11]

Clinical diagnosis of the cases and controls was based on a comprehensive eye examination that included slit lamp biomicroscopy, applanation tonometry, and gonioscopy (where corneal clarity permitted). Each PCG case had at least two of the following clinical features: increased corneal diameter (>12.0 mm) with raised IOP (>21 mm Hg) and/or Haab's striae, corneal edema/scar, and optic disc changes. The iris pattern was normal with developmental configuration in the angle due to anterior insertion of the iris; symptoms of epiphora and photophobia were corroborating features. Onset in all the patients was within 0 to 1 year of birth. We specifically looked for ocular and nonocular features that are considered diagnostic signs of ARS in the patient cohort. Patients presenting with any ocular features, such as posterior embryotoxon (a prominent, anteriorly displaced Schwalbe's line), adherence of iris strands to the Schwalbe's line, iris hypoplasia, focal iris atrophy with hole formation, corectopia, or ectropion uveae, with nonocular findings including developmental defects in the teeth and facial bones, pituitary and cardiac anomalies, oculocutaneous albinism, and redundant periumbilical skin were excluded. There were no signs of secondary glaucoma or other systemic features in these patients. All the cases and controls were independently evaluated and the diagnosis agreed on by two clinicians based on the inclusion–exclusion criteria.

The cases and controls were matched with respect to their ethnicity and geographical region of habitat. Two- to 4-mL blood samples from the probands along with their affected and normal relatives (when available) were collected by venipuncture with prior informed consent.

Screening of the *FOXC1* Gene

The genomic DNA was extracted from the blood samples according to standard protocols.[26] The entire coding region of *FOXC1* (Ensembl Gene ID: ENSG00000054598/ http://www.ensembl.org/[27]) was amplified with eight sets of overlapping primers as described in Table 1. The primers were designed using the Web-based Primer 3 software ((http://frodo.wi.mit.edu/[28]). A 25-μL PCR reaction was set up in a GeneAmp PCR system 9700 (Applied Biosystems, Inc. [ABI] Foster City, CA), using 50 to 100 ng of genomic DNA, 10× of PCR buffer, 200 μM of dNTPs, 5 to 12.5 picomoles of each primer and 1 unit of *Taq* polymerase (Banglore Genei, Bangalore, India). DMSO (10%) was added to the reaction mixture when necessary. The amplicons were

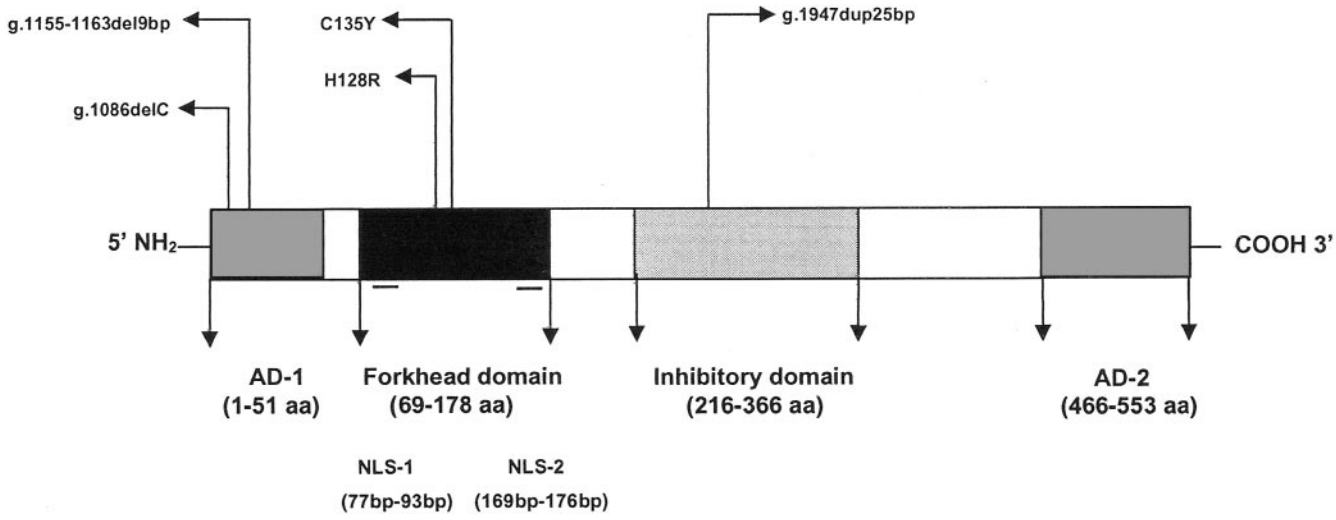

Figure 1. A schematic representation of the locations of the observed mutations in different domains of the FOXC1 coding regions (Ensembl[27] Human Gene ID: ENSG00000054598: AD-1: activation domain -1; AD-2: activation domain-2; NLS-1: nuclear localization signal 1; NLS-2: nuclear localization signal 2.

purified with PCR clean-up columns (Ultra Clean; Mo Bio Laboratories, Carlsbad, CA) and subjected to bidirectional sequencing on an automated DNA sequencer (model 3100; ABI), according to the manufacturer's guidelines. The data were analyzed with commercial software (Sequencing Analysis) also by ABI.

Validation of the Results

The observed variations were further confirmed by resequencing performed by an independent investigator who was masked to the genotype. Four of the five variations were also confirmed in cases and controls by PCR-based restriction digestion of the amplicons overnight at 37°C, using appropriate restriction enzymes. The digests were then fragmented on nondenaturing polyacrylamide gels and visualized by ethidium bromide staining.

Results

FOXC1 Mutations in PCG

Five different *FOXC1* mutations were observed in 5 (2.38%) of 210 cases (95% confidence interval [CI], 1.02–5.45). A schematic representation of the location of these mutations in *FOXC1* is provided in Figure 1, and the detailed clinical features of the patients with these mutations are provided in Table 2 (the electropherograms of all the observed mutations are provided in Supplementary Fig. S1, http://www.iovs.org/cgi/content/full/50/1/75/DC1). All the mutations were novel and had not been observed in ASD. The two missense mutations and the 25-bp duplication were observed in three sporadic cases, whereas the 1- and 9-bp deletions were present in two familial cases that were also heterozygous for a *CYP1B1* mutation.

Further details on the observed *FOXC1* mutations are presented in the following sections.

His128Arg. A missense heterozygous change at position g.1457 resulted in the replacement of Histidine (CAC) by Arginine (CGC) at codon 128 (H128R) in the FHD of *FOXC1* in a sporadic case (PCG209). The unaffected parents did not harbor the mutant *FOXC1* allele and the mutation in the proband seems to have occurred de novo (Supplementary Fig. S2, http://www.iovs.org/cgi/content/full/50/1/75/DC1). The patient had bilateral manifestation of the disease since birth (Table 2) and, compared with the patients in the other cases, had a relatively better reduction of IOP in both eyes.

Cys135Tyr. Another heterozygous change in the FHD of *FOXC1* was noted at position g.2713 that resulted in the replacement of Cysteine (TGC) by Tyrosine (TAC) at codon 135 (C135Y) in a sporadic case (PCG216). This change generated a gain of restriction site for the *Rsa*I enzyme. Similar to the PCG209 family, the unaffected parents did not harbor the mutant *FOXC1* allele, and the mutation in the probands seems to have occurred de novo (Supplementary Fig. S3, http://www.iovs.org/cgi/content/full/50/1/75/DC1). The proband had bilateral manifestation of the disease since birth (Table 2) and had a poor surgical outcome with IOPs of 28 and 34 mm Hg in the right and left eyes, respectively.

g.1086delC. A heterozygous deletion of a single base (C) at position g.1086 resulted in a frame shift at the 4th amino acid that led to a premature termination at the 43rd amino acid in the activation domain (AD)-1 of *FOXC1* in a consanguineous PCG family (PCG100). This change resulted in the loss of the restriction site for the *Pau*I enzyme and cosegregated in a proband who was also heterozygous for a *CYP1B1* mutation (R368H). His unaffected father and two siblings were heterozygous for the *CYP1B1* allele, whereas his mother harbored the heterozygous *FOXC1* allele (Fig. 2). She had optic atrophy leading to only light perception on inaccurate projection as well as superior oblique palsy in the right eye; the left eye was found to be normal. She did not manifest any other mutation in

Table 2. Clinical Features of Patients with *FOXC1* Mutations at Presentation

Patient ID	*FOXC1* Mutation	*CYP1B1* Mutation	Age at Onset	Corneal Diameter (mm)		IOP (mm Hg)		C:D Ratio		Visual Acuity		Corneal Changes	Treatment
				OD	OS	OD	OS	OD	OS	OD	OS		
PCG209	H128R	—	Since birth	15	14	32	30	0.9	0.9	20/40	20/40	Corneal edema with scar	TSCPC (OU)
PCG216	C135Y	—	Since birth	15	15	38	38	0.9	0.9	20/80	20/80	Corneal scar	TSCPC (OU)
PCG100	g.1086delC	R368H	Since birth	12	12.5	NA	NA	0.3	0.3	FFL	FFL	Corneal edema	TRAB & TRAB (OU)
PCG196	g.1155del9bp	R368H	Since birth	14	14	40	18	0.9	0.4	PLPR	20/20	Corneal scar with Haab's striae	Medication
PCG044	g.1947dup25bp	—	1 month	12	12	22	22	0.3	0.3	NA	NA	Corneal haze	TRAB & TRAB (OU)

TSCPC, transcleral-cyclo-photocoagulation; FFL, fixes and follows light; PLPR, perception of light on projection of rays; TRAB & TRAB, trabeculectomy & trabeculotomy; NA, not available.

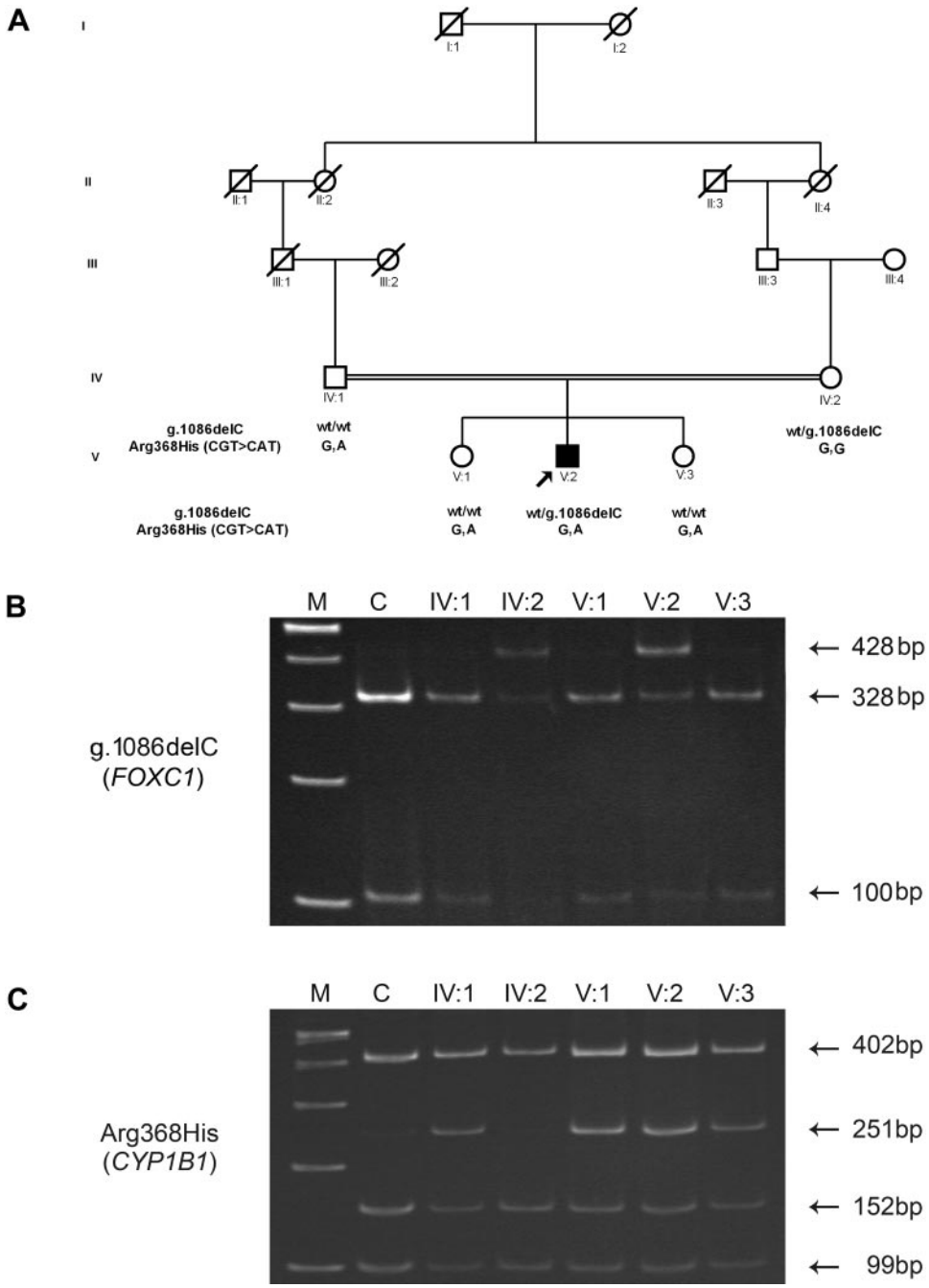

FIGURE 2. The segregation of *FOXC1* and *CYP1B1* mutations in family PCG100. The detailed pedigree of PCG100 (**A**) indicates the genotype of the individuals below their symbols at the *FOXC1* and *CYP1B1* loci. The DNA from the maternal grandparents of the probands (III:3 and III:4) was unavailable for analysis. (**B**) The segregation of the *FOXC1* allele based on a PCR-based restriction digestion on a 9% nondenaturing polyacrylamide gel. The g.1086delC mutation abolished a restriction site for *Pau*I that resulted in the generation of three fragments (428, 328, and 100 bp) in the proband (V:2) and his mother (IV:2) who were heterozygous for this mutation. The amplicon cleaved to two fragments (328 and 100 bp) in the wild-type (wt) in the father (IV:1) and two siblings (V:1 and V:3) of the probands and in an unrelated normal control (**C**). The segregation pattern of the heterozygous Arg368His mutation that abolished a restriction site for the *Taa*I enzyme in the proband, his father, and unaffected sibs were as described earlier.[13] M, 100-bp DNA ladder (GeneRuler; MBI Fermentas, Vilnius, Lithuania).

MYOC or *CYP1B1*. The proband had bilateral manifestation of the disease since birth, with elevated IOP and only light perception with inaccurate projection in both eyes (Table 2). Presently, his right eye had visual acuity of 20/60 and IOP of 16 mm Hg.

g.1155delCGCGGCGGC. In another consanguineous PCG family (PCG196), a heterozygous deletion of a 9 bp (CGCGGCGGC) at position g.1155 resulted in a frame shift at the 28th amino acid and led to the deletion of the alanine residues in the AD-1 domain of FOXC1. This change resulted in the loss of the restriction site for the *Not*I enzyme and was observed in the proband who was also heterozygous for a mutant *CYP1B1* allele (R368H). His unaffected parents and a sister also harbored the heterozygous *FOXC1* allele (Fig. 3). In addition, the mother was heterozygous for the *CYP1B1* allele (R368H), similar to the proband, but did not manifest any signs of glaucoma or other ocular or systemic diseases. The g.1155del9bp change was also observed in 0.95% (3/314) normal chromosomes. The proband had a unilateral manifestation of the disease in the right eye since birth (Table 2) and had visual acuity of no light perception and no reduction in IOP.

g.1927dupTCAGCCTGGACGGTGCGGATTCCGC. A heterozygous duplication of 25 bp (TCAGCCTGGACGGTGCGGATTCCGC) at position g.1927 resulted in a frame shift at the 291st amino acid leading to premature termination after the 313th amino acids in the inhibitory domain of FOXC1 in a sporadic PCG case (PCG044). The cosegregation of the mutation in this family could not be analyzed, as a DNA sample from the proband's mother was unavailable for analysis; his father did not harbor the mutant allele (Supplementary Fig. S4, http://www.iovs.org/cgi/content/full/50/1/75/DC1). The proband had bilateral manifestation of the disease since 1 month of age (Table

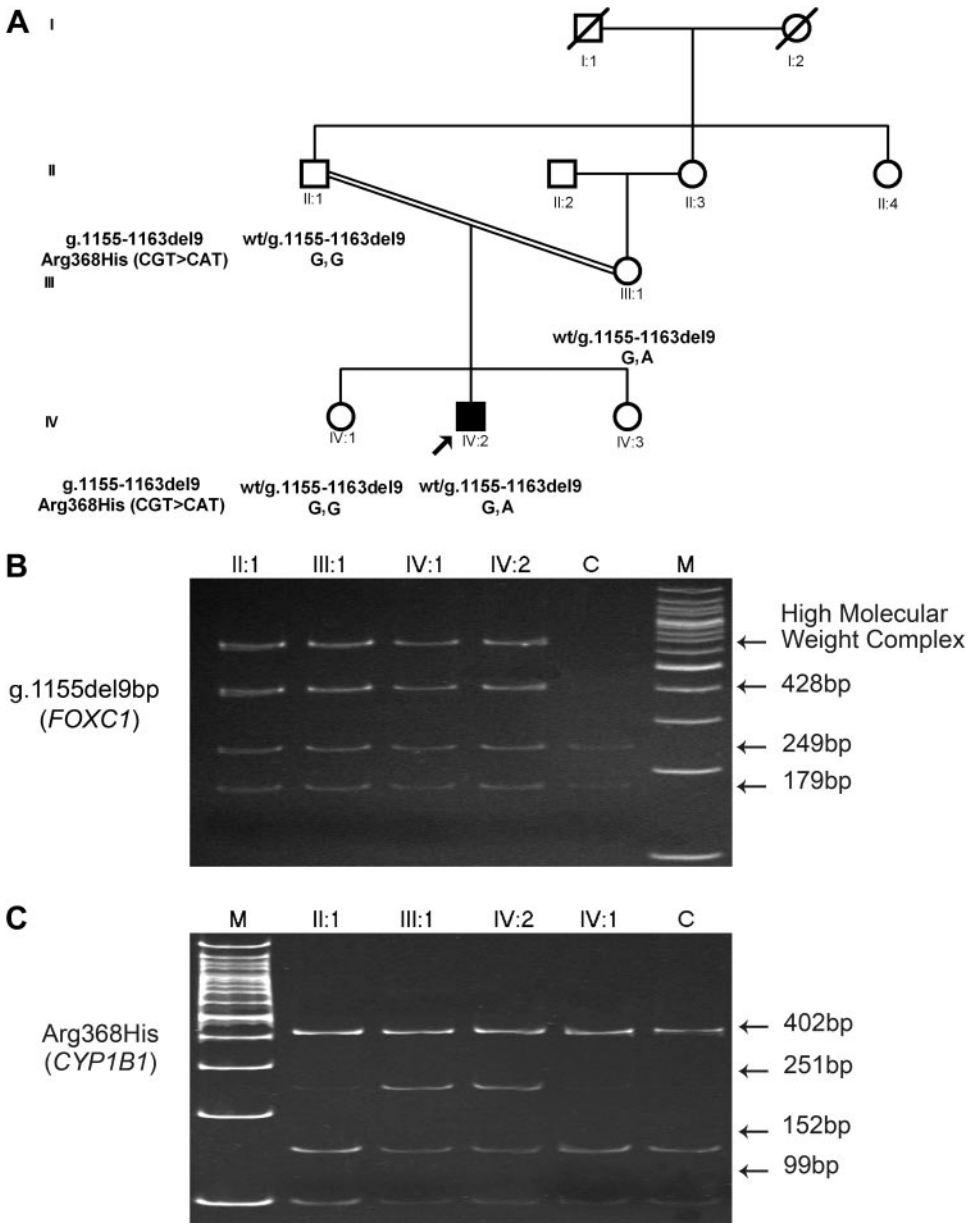

FIGURE 3. The segregation of *FOXC1* and *CYP1B1* mutations in PCG196 family. The detailed pedigree of PCG196 family (**A**) indicates the genotype of the individuals below their symbols at the *FOXC1* and *CYP1B1* loci. The DNA from the maternal grandparents of the proband (II:2 and II:3) and his unaffected sib (IV:3) were unavailable for analysis. (**B**) Demonstrates the segregation of the *FOXC1* allele based on a PCR-based restriction digestion on a 9% nondenaturing polyacrylamide gel. The g.1155del9bp mutation abolished a site for the *Not*I enzyme and generated three fragments of 428, 249, and 179 bp in the proband (IV:2), his unaffected parents (II:1 and III:1), and a sib (IV:1) who were heterozygous for the mutation. The amplicon cleaved to two fragments (249 and 179 bp) in the wild-type (wt) in an unrelated normal control (C). The segregation pattern of the heterozygous Arg368His mutation that abolished a restriction site for *Taa*I enzyme in the probands and his mother were as described earlier[13] (**C**). M, 100 bp DNA ladder (GeneRuler; MBI Fermentas, Vilnius, Lithuania).

2). The last visual acuity was 20/20 in both eyes with IOPs of 12 and 10 mm Hg in the right and left eyes, respectively.

Except for g.1155del9bp, none of the *FOXC1* mutations were observed in the 157 unaffected control subjects. Multiple-sequence alignment indicated that the wild-type residues of the missense changes (His128Arg and Cys135Tyr) were highly conserved across FOX families in different species (Fig. 4). Both the missense mutations seemed to have originated de novo and were absent in the parents of the probands in the PCG209 and PCG216 families. The possibility of disputed parentage was ruled out by screening the parents and the probands of these two families with 48 microsatellite markers chosen randomly from eight chromosomes. These comprised highly polymorphic dinucleotide repeat markers from the Genethon linkage map (www.genethon.fr; provided in the public domain by the French Association against Myopathies, Evry, France) that were selected from the ABI Linkage panel MD-10 and genotyped as per the manufacturer's protocol (ABI). All the markers exhibited a perfect Mendelian segregation in these two families (data not shown).

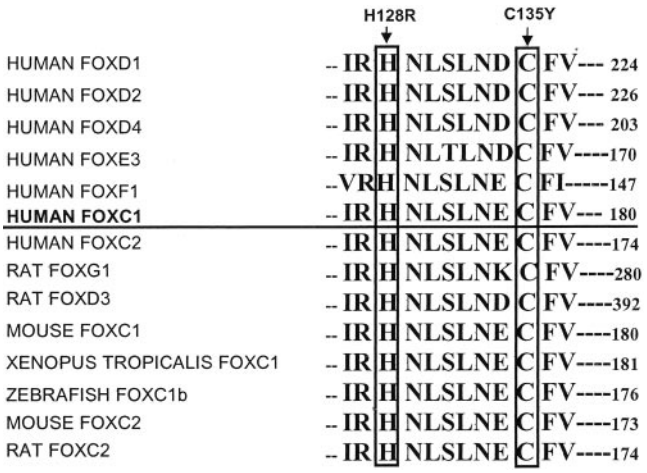

FIGURE 4. Multiple sequence alignment of different FOX protein across humans and other species indicates a high degree of conservation at the H128 and C135 residues.

TABLE 3. Distribution of Genotypes and Odds Ratios for the Two *FOXC1* Polymorphisms in PCG Cases and Control Subjects

FOXC1 Polymorphism	Genotype	Cases (n = 210)	Controls (n = 110)	OR (95% CI)	*P*
GGC375ins	GGC_6,GGC_6*	78 (37.1%)	36 (32.7%)	1	—
GGC375ins	GGC_6,GGC_7†	98 (46.7%)	58 (52.7%)	0.78 (0.47–1.30)	0.170
GGC375ins	GGC_7,GGC_7‡	34 (16.2%)	16 (14.5%)	0.98 (0.48–2.00)	0.479
GGC447ins	GGC_7,GGC_7*	97 (46.2%)	52 (47.3%)	1	—
GGC447ins	GGC_7,GGC_8†	86 (40.9%)	44 (40.0%)	1.05 (0.64–1.72)	0.290
GGC447ins	GGC_8,GGC_8‡	27 (12.9%)	14 (12.7%)	1.03 (0.50–2.14)	0.367

* No insertion of GGC.
† Heterozygous insertion of GGC.
‡ Homozygous insertion of GGC.

FOXC1 Polymorphisms in PCG

Two polymorphisms leading to an insertion of one copy of "GGC" at positions g.2197 (GGC375ins) and g.2413 (GGC447ins) in the second activation domains and reported in earlier studies[29,30] were observed among cases and controls. In the GGC375ins polymorphism, there was an insertion of an additional GGC repeat (GGC_7) from the wild-type allele (GGC_6), and in the second polymorphism GGC475ins, there was an addition of GGC (GGC_8) from the wild-type (GGC_7) alleles. There were no differences in the distribution of genotype frequencies for these two polymorphisms across PCG cases and controls (Table 3). We also generated haplotypes with these two variants in cases and controls and their estimated frequencies are provided in Table 4. There was no significant association of any of the four haplotypes with PCG.

DISCUSSION

The *FOXC1* gene has been implicated in ASD, particularly in ARS worldwide, and a wide spectrum of mutations have been reported across multiple studies.[29–34] Some of these studies showed the involvement of *FOXC1* in a few cases of congenital or early-onset glaucoma associated with ASD and/or other ocular and nonocular diseases.[15,18,25,29–34] *FOXC1* null ($Foxc1^{-/-}$) mice and heterozygous ($Foxc1^{+/-}$) mice (from certain genetic backgrounds) exhibit malformations in the anterior segment of various degrees of severity. These malformations also lead to abnormalities in the ocular drainage structures.[23] Despite these indications, *FOXC1* has not been extensively explored as a candidate gene in cases of classic PCG. To the best of our knowledge, this is perhaps the first study to report the involvement of *FOXC1* in large cohort of PCG (n = 210) cases.

TABLE 4. Estimated Haplotype Frequencies for the Two *FOXC1* SNPs among PCG Cases and Control Subjects

Haplotypes	% Cases	% Controls	*P*
GGC_6-GGC_7*	38.4	39.4	0.800
GGC_7-GGC_7†	28.2	27.8	0.926
GGC_6-GGC_8‡	21.8	19.7	0.530
GGC_7-GGC_8§	11.6	13.1	0.595

* No insertion of GGC at either loci.
† Insertion of GGC in the first locus and no insertion at the second locus.
‡ No insertion of GGC at the first locus but insertion at the second locus.
§ Insertion of GGC at both the loci.

The frequency of *FOXC1* mutations in the present PCG cohort and in earlier studies on ARS is provided in Table 5. Although the proportion of *FOXC1* mutations in the present study is lower than in ARS,[29–34] some novel mutations were identified in PCG. An earlier study that screened *FOXC1* in a small number of PCG cases (n = 6) by single-strand conformation polymorphism (SSCP) did not identify any mutation (95% CI, 0–39.0).[15] This inability could also be attributable to the low rate of mutation detection by SSCP.[35] Apart from the mutations, the two *FOXC1* polymorphisms GGC375ins and GGC447ins were present in almost equal frequencies among the PCG cases and controls (Table 3) similar to earlier studies on ARS.[29,30]

The overall spectrum of *FOXC1* mutations worldwide indicates a strong association with ASD particularly with ARS (Table 6). Before this study, ~37 mutations were identified in *FOXC1*, further highlighting its allelic heterogeneity.[15,29–34,36–44] The majority (26; 70.3%) of these mutations were located in the FHD. Eleven mutations resulted in frame shifts leading to protein truncation. The patients harboring these mutations exhibited ocular features similar to ARS; some of them also manifested certain nonocular features (Table 6).

TABLE 5. Distribution of *FOXC1* Mutations in ARS and Other Glaucoma Phenotypes Worldwide

Populations	Cases (n)	Phenotype	Cases with *FOXC1* Mutation (n (%) [95% CI])	Cases without *FOXC1* Mutation (n (%) [95% CI])
Brazil[30]	8	ARS+DG*	2 (25.0) [7.1–59.1]	6 (75.0) [40.9–92.8]
European[15]	19	ARS+PCG	4 (21.1) [8.5–43.3]	15 (78.9) [56.7–91.5]
Germany[31]	13	ARS	7 (53.8) [29.1–76.8]	6 (46.2) [23.2–70.8]
India[32]	9	ARS	3 (33.3) [12.1–54.6]	6 (66.7) [35.4–87.9]
Japanese[33]	6	ARS	4 (66.7) [29.9–90.3]	2 (33.3) [9.7–70.0]
United States[29]	21	ARS	3 (14.3) [4.9–34.6]	18 (85.7) [65.4–95.0]
United States[34]	70	ARS	9 (12.9) [6.9–22.7]	61 (87.1) [77.3–93.0]
India [present study]	210	PCG	5 (2.4) [1.0–5.4]	205 (97.6) [94.5–99.0]

* Developmental glaucoma.

TABLE 6. The Spectrum of *FOXC1* Mutations Identified in Different Anterior Segment Phenotypes Worldwide

SI No.	Genomic DNA Position	Position in the Protein	Type of Mutation	Amino Acid Change	Ocular Features	Nonocular Features	References
1	g.1078CAG>TAG	AD-1	Nonsense	Q2X	ARA	—	32
2	**g.1086delC**	**AD-1**	**Frameshift**		**PCG**	—	**Present study**
3	g.1100-1121ins22	AD-1	Frameshift		Axenfeld anomaly	—	33, 34
4	g.1141CAG>TAG	AD-1	Nonsense	Q23X	ARS	—	36
5	**g.1164del9bp**	**AD-1**	**Frameshift**		**PCG**	—	**Present study**
6	g.1167–1176del10	AD-1	Frameshift		ARA	—	29
7	g.1173–1182del10	AD-1	Frameshift		Axenfeld anomaly	—	34
8	g.1190–1197del8	AD-1	Frameshift		RA	—	34
9	g.1217TCG>TAG	AD-1	Nonsense	S48X	Iridocorneal adhesions, posterior embryotoxon	—	31
10	g.1227–1236del11	AD-1	Frameshift		RA	—	15
11	g.1309CCG>ACG	FHD	Missense	P79T	ARS	—	37
12	g.1310CCG>CTG	FHD	Missense	P79L	RA	—	34
13	g.1310CCG>CGG	FHD	Missense	P79R	Iridocorneal adhesions, posterior embryotoxon	Micrognathia	31
14	g.1312–1315insC	FHD	Frameshift		ARA	—	34
15	g.1319AGC>ACC	FHD	Missense	S82T	Posterior embryotoxon, congenital glaucoma	—	29
16	g.1327GCG>CCG	FHD	Missense	A85P	Hazy megalocornea, posterior embryotoxon, iris hypoplasia, corectopia, early onset glaucoma	Atrial septal defects, aortic stenosis, pulmonary stenosis	38
17	g.1328CTC>TTC	FHD	Missense	L86F	Iris hypoplasia, iridocorneal adhesions in the angle, mild corectopia, congenital glaucoma	Short stature, obesity, myocardial infarction, dental abnormalities	39
18	g.1335ATC>ATG	FHD	Missense	I87M	Corectopia, glaucoma, goniodysgenesis, iris strands, posterior embryotoxon	Deafness, heart anomalies	29
19	g.1336insG		Frameshift		ARA	—	33
20	g.1346ATC>ACC	FHD	Missense	I91T	ARA	—	40
21	g.1346ATC>AGC	FHD	Missense	I91S	Iris hypoplasia with severe early onset glaucoma	—	33
22	g.1409TTC>TCC	FHD	Missense	F112S	RA and iris hypoplasia	—	15, 41
23	g.1418TAC>TCC	FHD	Missense	Y115S	Iridocorneal adhesions, posterior embryotoxon, iris hypoplasia, megalocornea	Mild deafness	31
24	g.1441CAG>TAG	FHD	Nonsense	Q123X	ARA, Hazy cornea, edema	—	32
25	g.1452ATC>ATG	FHD	Missense	1126M	Axenfeld anomaly and glaucoma	—	15
26	g.1454CGC>CAC	FHD	Missense	R127H	Iris hypoplasia with severe early onset glaucoma	—	33
27	**g.1457CAC>CGC**	**FHD**	**Missense**	**H128R**	**PCG**	—	**Present study**
28	g.1462CTC>TTC	FHD	Missense	L130F	ARS	—	42
29	g.1466TCG>TTG	FHD	Missense	S131L	RA and glaucoma	—	15
30	**g.1478TGC>TAC**	**FHD**	**Missense**	**C135Y**	**PCG**	—	**Present study**
31	g.1511–1527del17	FHD	Frameshift		Posterior embryotoxon, congenital glaucoma	Hearing loss, hypertension	38
32	g.1520GGC>GAC	FHD	Missense	G149D	Iridocorneal adhesions, posterior embryotoxon, corectopia	Hypospadia, heart defect	31
33	g.1530TGG>TGA	FHD	Nonsense	W152X	ARA	—	30
34	g.1555ATG>GTG	FHD	Missense	M161V	Iridocorneal adhesions, posterior embryotoxon, iris hypoplasia	Umbilicus, middle ear deafness	31
35	g.1556ATG>AAG	FHD	Missense	M161K	ARA	—	32, 43
36	g.1567GGC>CGC	FHD	Missense	G165R	Iris stroma hypoplasia, posterior embryotoxon, corectopia, glaucoma	Dental abnormalities	44
37	g.1580CGG>CCG	FHD	Missense	R169P	Iris hypoplasia, hypertelorism, corneal opacity, abnormal pupillary function	Hearing loss	44
38	g.1792–1793delCT	Inhibitory domain	Frameshift		ARA	—	30
39	g.1814delG	Inhibitory domain	Frameshift		Posterior embryotoxon, iris hypoplasia, iridocorneal adhesion	Hypertelorism, umbilicus	31
40	**g.1947dup25bp**	**Inhibitory domain**	**Frameshift**		**PCG**	—	**Present study**
41	g.2585delT	AD-2	Frameshift		Iris hypoplasia	—	31
42	g.2656delA	AD-2	Frameshift		Axenfeld anomaly	—	34

RA, Rieger anomaly. The mutations shown in bold are those observed in the present study.

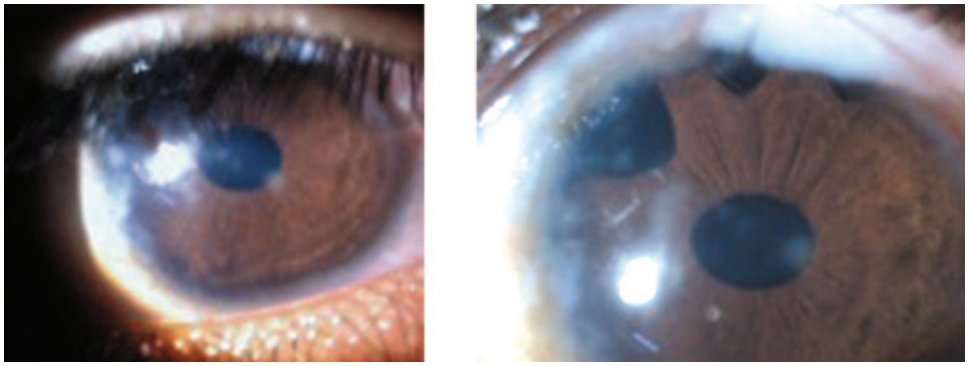

FIGURE 5. *Left*: clinical photograph of the right eye of a patient with PCG (family PCG100) with the *FOXC1* mutation g.1086delC, showing the postsurgical iris pattern. *Right*: magnified view of the same eye exhibiting the locations of surgical iridectomies performed multiple times. Pachymetry in this eye revealed a central corneal thickness of 540 μm.

The five PCG cases that harbored the mutant *FOXC1* allele neither manifested any ocular signs suggestive of anterior segment anomalies (Fig. 5) nor had any other extraocular features (Table 6). We also screened a cohort of patients with ARS (n = 28), who were diagnosed based on the inclusion criteria described earlier,[32] for these five PCG-associated *FOXC1* mutations. None of the patients with ARS harbored any of these mutations (95% CI, 0–12.1). Whether these mutations are specific to PCG, needs further evaluation on large and extensive PCG cohorts from different ethnic backgrounds.

It has been demonstrated through in vitro experiments that *FOXC1* functionally interacts with another transcription factor *PITX2* in a common biochemical pathway and that *PITX2* regulates *FOXC1* gene dosage that may underlie ASD phenotypes.[45] Based on this, it is tempting to speculate that *FOXC1* gene dosage changes may also contribute to PCG pathogenesis through some common mechanisms.

A perfect genotype–phenotype correlation could not be established, as the clinical presentations among patients with PCG harboring *FOXC1* mutations were not significantly different from those who did not harbor them. Compared with our previous studies of patients with PCG who harbored *CYP1B1* mutations,[11] we did not observe any major differences in disease severity and progression among patients who manifested *FOXC1* mutations in the present cohort. Based on an extensive genotype–phenotype correlation, it was suggested that patients with ARS with *FOXC1* mutations had a relatively lower incidence of glaucoma development than those with duplications in the *FOXC1* gene.[18,25] Although such a distinction was not observed in the present study, all the patients with *FOXC1* mutations had classic PCG with a severe phenotype of raised IOP and megalocornea at presentation (Table 2).

The involvement of *FOXC1* in PCG cases unlinked to *CYP1B1* alone in the present cohort (2.4%) was relatively lower compared to our previous study in this category on *MYOC* (5.5%). Although our earlier patient cohort had a smaller sample size (n = 72), the mutation frequency of *MYOC* in PCG was almost similar to the global frequency in POAG.[13]

We had also demonstrated a possible digenic interactions of *MYOC* and *CYP1B1* in PCG[13] similar to that observed in juvenile open-angle glaucoma.[14] A similar scenario was observed in the present cohort with respect to the presence of double heterozygotes in *FOXC1* and *CYP1B1* in the proband of a consanguineous PCG100 family. Both his parents were heterozygous for either of the mutant alleles; his unaffected sibs also harbored the R368H (*CYP1B1*) allele and did not manifest any signs of glaucoma (Fig. 2). The association of the heterozygous *FOXC1* allele (g.1086delC) with optic atrophy in his mother requires further investigation. Likewise, the proband in the other consanguineous family (PCG196) was double heterozygous for both the *FOXC1* and *CYP1B1* allele, similar to his unaffected mother (Fig. 3). His unaffected father and brother also manifested the heterozygous *FOXC1* allele (g.1155del9bp). Although the *CYP1B1* allele (R368H) was not observed among the 157 unaffected controls, the *FOXC1* allele was found in 0.95% of control chromosomes. Thus a clear-cut digenic inheritance of *FOXC1* and *CYP1B1* could not be established in these PCG cases unlike our previous study on *MYOC*.[13]

Multiple studies worldwide have associated ARS, an autosomal dominant disorder, with a single mutant allele of *FOXC1*.[15,29–44] Whether a heterozygous *FOXC1* mutation is sufficient to cause autosomal recessive PCG (as observed in the present study) is speculative. However, such speculations can be addressed only when more data on *FOXC1* mutations from diverse PCG cohorts worldwide become available, followed by functional characterization of the mutant protein.

In summary, the present study provides an overview of the involvement of *FOXC1* in a large PCG cohort that is either partially (heterozygous) or completely unlinked to *CYP1B1*. Five novel mutations were observed in PCG thereby adding to the overall mutation spectrum of *FOXC1*. The involvement of the double heterozygous variants *FOXC1* and *CYP1B1* in two cases was interesting, but their role in disease causation is yet to be established. Finally, our data indicated a limited role of *FOXC1* in PCG that suggests other causative loci, which are yet uncharacterized in the disease's pathogenesis.

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