Analysis of *CYP1B1* in pediatric and adult glaucoma and other ocular phenotypes

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Purpose: The *CYP1B1* gene encodes an enzyme that is a member of the cytochrome P450 superfamily. Mutations in *CYP1B1* have been mainly reported in recessive pediatric ocular phenotypes, such as primary congenital glaucoma (PCG) and congenital glaucoma with anterior segment dysgenesis (CG with ASD), with some likely pathogenic variants also identified in families affected with adult-onset primary open angle glaucoma (POAG).

Methods: We examined *CYP1B1* in 158 pediatric patients affected with PCG (eight), CG with ASD (22), CG with other developmental ocular disorders (11), juvenile glaucoma with or without additional ocular anomalies (26), and ASD or other developmental ocular conditions without glaucoma (91); in addition, a large cohort of adult patients with POAG (193) and POAG-negative controls (288) was examined.

Results: Recessive pathogenic variants in *CYP1B1* were identified in two PCG pedigrees, three cases with CG and ASD, and two families with CG and other ocular defects, such as sclerocornea in one patient and microphthalmia in another individual; neither sclerocornea nor microphthalmia has been previously associated with *CYP1B1*. Most of the identified causative mutations are new occurrences of previously reported pathogenic alleles with two novel variants identified: a c.1325delC, p.(Pro442Glnfs*15) frameshift allele in a family with PCG and a c.157G>A, p.(Gly53Ser) variant identified in a proband with CG, Peters anomaly, and microphthalmia. Analysis of the family history in the *CYP1B1*-positive families revealed POAG in confirmed or presumed heterozygous relatives in one family with PCG and two families with ASD/CG; POAG was associated with the c.1064_1076del, p.(Arg355Hisfs*69) allele in two of these pedigrees. Screening of an unrelated POAG cohort identified the same c.1064_1076del heterozygous allele in one individual with sporadic POAG but not in age- and ethnicity-matched POAG-negative individuals. Overall, there was no significant enrichment for mutant alleles in *CYP1B1* within the POAG cases compared to the controls.

Conclusions: In summary, these data expand the mutational and phenotypic spectra of *CYP1B1* to include two novel alleles and additional developmental ocular phenotypes. The contribution of *CYP1B1* to POAG is less clear, but loss-of-function variants in *CYP1B1*, especially c.1064_1076del, p.(Arg355Hisfs*69), may be associated with an increased risk for POAG.

Glaucoma, a degenerative optic neuropathy, represents an important cause of blindness worldwide [1,2]. Primary congenital glaucoma (PCG) is characterized by onset in newborns or diagnosis within the first 2 years of life (also referred to as infantile) and the absence of visible structural ocular anomalies [3]. In many cases, congenital glaucoma is accompanied by anterior segment dysgenesis (ASD), such as iris hypoplasia, iridocorneal adhesions, or Peters anomaly (CG with ASD), thus indicating a defect in the embryonic development of ocular structures. Primary open angle glaucoma (POAG) is the most common form of glaucoma characterized by adult onset and the absence of visible ocular defects. Congenital glaucomas (primary or associated with ASD) are typically inherited as Mendelian traits (autosomal recessive or dominant) while POAG demonstrates a complex inheritance pattern in most pedigrees [4-7].

CYP1B1 (OMIM# 601771) is a member of the cytochrome P450 superfamily and encodes a monooxygenase involved in the metabolism of a broad range of endogenous and exogenous substrates. Mutations in *CYP1B1* have been shown to

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cause various glaucoma phenotypes [7]. The role of *CYP1B1* in PCG is well established with homozygous or compound heterozygous missense or truncating variants reported in more than 600 patients [7-10]. Homozygous or compound heterozygous mutations in *CYP1B1* have also been reported in patients with ASD, including Peters anomaly, Axenfeld-Rieger anomaly, ectropion uveae with partial aniridia, and even complete aniridia [11-18]; the majority of these patients also had congenital glaucoma. Significant allelic heterogeneity exists with more than 150 distinct mutations already reported [9,10]. Phenotypic heterogeneity is also seen with juvenile open angle glaucoma (JOAG)/POAG occasionally reported in individuals with homozygous pathogenic variants in *CYP1B1* [19,20]. Heterozygous variants in *CYP1B1*, primarily missense alleles, have been reported in sporadic and familial POAG cases [21-30].

CYP1B1 mutant proteins demonstrate decreased enzymatic activity or decreased protein stability [24,31-34]. Studies in mice revealed abnormalities in the trabecular meshwork of *Cyp1b1*-deficient animals, including irregular collagen distribution, increased oxidative stress, and decreased levels of periostin (Postn), a secreted extracellular matrix protein expressed in collagen-rich tissues, including the trabecular meshwork. Studies of trabecular meshwork cells from human patients with glaucoma confirmed increased oxidative stress and decreased levels of Postn, suggesting a possible mechanism for the glaucoma phenotype in patients with mutations in *CYP1B1* [35]. Recent functional characterization of missense mutations in *CYP1B1* identified that variants associated with PCG typically affected the metabolism of retinol, thus disrupting the level of retinoic acid that is known to be critical for ocular development, while variants associated with POAG affected the metabolism of 17β-estradiol, which may contribute to POAG through *MYOC* (OMIM# 601652) overexpression or increased levels of reactive oxygen species and apoptosis [36,37]. Of note, some variants affected both processes and were associated with both types of glaucoma while other variants specifically affected only one or the other. To further define the contribution of *CYP1B1* to human glaucoma phenotypes, as well as to investigate its possible role in other ocular conditions, we screened *CYP1B1* in patients with various developmental ocular phenotypes, as well as a cohort affected with POAG.

METHODS

Human subjects: This human study was approved by the Institutional Review Board of the Children's Hospital of Wisconsin and adhered to the tenets of the Declaration of Helsinki and ARVO guidelines. Written informed consent

was obtained for every subject; in addition, informed consent was obtained for all individual participants whose photographs are included. We screened DNA from 158 probands with developmental ocular conditions for variants in *CYP1B1*, including eight with PCG, 22 with ASD and congenital glaucoma, 11 with other developmental ocular disorders (including microphthalmia, anophthalmia, coloboma, sclerocornea, and cataract) and congenital glaucoma, three with isolated juvenile glaucoma, 19 with ASD/juvenile glaucoma, four with other developmental ocular disorders and juvenile glaucoma, 50 with ASD without glaucoma, and 41 with other developmental ocular phenotypes without glaucoma. For this study, PCG was used for congenital glaucoma without visible structural ocular anomalies while ASD included diagnoses such as Axenfeld-Rieger anomaly, Peters anomaly, iris hypoplasia, pupil anomalies, and generalized anterior segment dysgenesis that did not fit a specific diagnosis. Patients were from a variety of racial and ethnic backgrounds. Many patients were additionally screened for mutations in *PITX2* (OMIM 601542), *FOXC1* (OMIM 601090), *FOXE3* (OMIM 601094), *BG3LCT* (OMIM 610308), and other genes with no pathogenic alleles identified [38-40]. The full *CYP1B1* coding sequence was obtained for all probands; all available family members were screened for the specific mutations identified in the proband.

In addition, 193 adults with POAG diagnosed by their ophthalmologists from the Marshfield Clinic Personalized Medicine Research Project (PMRP) were screened. The PMRP project was reviewed and approved by the Institutional Review Board of Marshfield Clinic, and all participants provided written informed consent. This population has been described previously [41]. One case was diagnosed in childhood (12.2 years); the age of diagnosis for the remainder ranged from 29.8 to 86.8 years with an average age of 61.2 years. The population was primarily Caucasian (184); 115 cases were female, and 78 were male. In addition, 288 controls from the PMRP were screened. Controls from the PMRP all had normal ophthalmic exams within the past 3 years. Age ranged from 29.4 to 84.8 years with an average age of 55.3 years. The control population was primarily Caucasian (280); 169 cases were female, and 119 were male. Variant frequencies in the general population were obtained from the Exome Aggregation Consortium [42].

CYP1B1 mutation screen: Blood or buccal samples were obtained from each participant and genomic DNA was extracted using Qiagen reagents/kits (Quiagen, Valencia, CA) and standard protocols. Whenever possible, samples were extracted within 24-48 h of draw and refrigerated prior to extraction. The entire coding region and the exon–intron

junctions of *CYP1B1* (reference sequence NM_000104.3) were screened with direct DNA sequencing of PCR products in the cases and controls. The *CYP1B1* coding region was amplified using the following primers: set 1 forward, 5'- TCT CCA GAG AGT CAG CTC CG-3', and set 1 reverse, 5'- GGG TCG TCG TGG CTG TAG-3', and set 2 forward, 5'- ATG GCT TTC GGC CAC TAC T-3', and set 2 reverse, 5'- GAT CTT GGT TTT GAG GGG TG-3', and set 3 forward 5'- TCC CAG AAA TAT TAA TTT AGT CAC TG-3' and set 3 reverse 5'- TAT GGA GCA CAC CTC ACC TG-3'. PCR products were sequenced in both directions with standard conditions and 20% betaine using Big Dye Terminator v3.1 (Applied Biosystems, Foster City, CA) with 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA). Chromatograms were examined manually and using Mutation Surveyor software (SoftGenetics, State College, PA). All initially identified changes were confirmed with additional independent PCR and sequencing experiments. In silico analysis of the effect of missense mutations was performed using SIFT, PolyPhen2, MutationTaster, MutationAssessor, and FATHMM prediction algorithms accessed through the SNP & Variation Suite software package (SVS; Golden Helix, Bozeman, MT) using data from dbNSFP 2.9 [43].

RESULTS

Mutations in CYP1B1 in pediatric phenotypes: Homozygous or compound heterozygous pathogenic variants in *CYP1B1* were identified in seven families affected with the following phenotypes: PCG (Patients 1, 2), congenital glaucoma with ASD (Patients 3–5), sclerocornea and congenital glaucoma (Patient 6), and microphthalmia, Peters anomaly, and congenital glaucoma (Patient 7; Table 1, Figure 1, Figure 2). Eight pathogenic variants were identified; two of these alleles were novel (c.1325delC p.(Pro442Glnfs*15) and c.157G>A p.(Gly53Ser); Table 1), and the remaining six have each been previously reported as causative mutations in congenital glaucoma [9,10]. All of the variants are extremely rare (<0.09% allele frequency) or novel with the exception of the c.1103G>A p.(Arg368His) variant, which was present in 0.3% of Caucasian and 2.9% of South Asian alleles in ExAC with ten homozygotes present in this general population database. This variant has been seen more than 90 times in patients with PCG [9,10], and incomplete penetrance has been reported in multiple families with homozygous or compound heterozygous missense mutations in *CYP1B1* [12,44,45].

The novel frameshift mutation, c.1325delC, p.(Pro442Glnfs*15), identified in Patient 2, is a truncating variant located within the meander region of the protein; this allele cosegregates with the disease phenotype in the affected

family and is seen in only 1 in 1,6512 South Asian alleles [42]. The novel missense mutation, c.157G>A, p.(Gly53Ser), seen in Patient 7, affects a highly conserved glycine within the hinge region of CYP1B1 and is predicted to affect protein function by all five functional effect prediction programs; this allele is present in trans with a second pathogenic mutation and is not seen in the general population [42].

Two patients presented with features not previously described for *CYP1B1*. Patient 6 presented with bilateral congenital glaucoma with sclerocornea and a homozygous c.182G>A, p.(Gly61Glu) mutation previously reported in patients with PCG [10]. Patient 7, with the novel c.157G>A, p.(Gly53Ser) and previously reported c.1405C>T, p.(Arg469Trp) mutations, is affected with microphthalmia and opacified cornea in the right eye, anterior staphyloma of the left eye, bilateral type II Peters anomaly, and bilateral congenital glaucoma. He underwent numerous surgeries, including bilateral cornea transplant, lensectomy, and retinal detachment repair, but ultimately lost vision in both eyes.

A family history of PCG was present in two cases (Patients 1 and 2); the identified variants were shown to cosegregate with the phenotype in both families (Figure 2A,B). In all but one family, both parental samples were available for testing. All unaffected parents were found to be heterozygous for the variant seen in their child, and one affected parent (Family 2) was homozygous for the same mutation seen in his child. Three of the seven probands with congenital glaucoma in this study (Patients 1, 3, and 5) have a relative in their grandparents' or great-grandparents' generation reported to have POAG (age of diagnosis ranged from 44 to 60 years), and an additional proband (Patient 4) has a grandparent with vision loss later in life of unknown cause. In two families, the c.1064_1076del mutation appears to be involved with the mutation confirmed in an affected relative in one family and present in the daughter of the affected relative in the other (Figure 2A,C).

No homozygous or compound heterozygous pathogenic mutations were seen in patients with juvenile glaucoma with or without ASD/other developmental ocular conditions (26), ASD without glaucoma (50), or other developmental ocular conditions without glaucoma (41). Three heterozygous variants of uncertain significance (c.241T>A, p.(Tyr81Asn), c.1328C>G, p.(Arg443Gly), and c.1462G>C, p.(Ala488Pro)) were identified in patients with variable anterior segment defects or microphthalmia (Appendix 1). Finally, the previously reported c.685G>A, p.(Glu229Lys) heterozygous variant was identified in five pediatric probands without glaucoma (1.6% allele frequency) matching the overall allele frequency of 1.4% in the general population (ExAC), adding further

evidence that this is a benign polymorphism. Previous functional analysis of this variant also suggested that this variant did not affect CYP1B1 activity [37].

Mutations in CYP1B1 in POAG: Screening of 193 POAG cases identified an individual who was heterozygous for the c.1064_1076del, p.(Arg355Hisfs*69) allele that was found to

be associated with POAG in two pediatric pedigrees reported here. In addition, three other rare heterozygous variants were identified: c.241T>A, p.(Tyr81Asn) and c.1328C>G, p.(Arg443Gly), which were both previously reported in POAG [21,46], and a novel c.35C>T (p.Pro12Leu) variant (Table 2). Two of these variants, p.(Arg443Gly) and (p.Pro12Leu), were predicted to be benign by all five prediction algorithms, and

Patient#	Race/ ethnicity	*CYP1B1* mutation[a]	Functional prediction[b]	Allele frequency[c]	# homo- zygote controls[d]	Eye	Family history
1	Caucasian (U.S./ Spain)	c.1064_1076del p.(Arg355Hisfs*69) (mat)	Premature truncation	NP	0	Bilateral PCG	Brother with PCG; several relatives with **adult-onset glaucoma**; distant cousin with PCG
		c.1159G>A p.(Glu387Lys) (pat)	Damaging by 5/5 (S, PP, MT, MA, F)	36/64286	0		
2	S. Asian (Pakistan)	homozygous **c.1325delC p.(Pro442Glnfs*15)** (mat + pat)	Premature truncation	1/16512	0	Bilateral PCG	Extensive family history of PCG
3	Hispanic (U.S.)	c.535delG p.(Ala179Argfs*18) (pat)	Premature truncation	0/1080	0	Bilateral CG, right form fruste Axenfeld anomaly (synechia and pupil eccentricity)	Maternal grand-mother with **adult-onset glaucoma**
		c.1064_1076del p.(Arg355Hisfs*69) (mat)	Premature truncation	NP	0		
4	Caucasian (Turkey)	homozygous c.1103G>A p.(Arg368His) (mat + pat)	Damaging by 4/5 (S, PP, MT, MA)	184/63292	9 SA 1 Eu	Bilateral CG, iris hypoplasia, posterior embryotoxon	Consanguinity; maternal grand-mother with vision loss later in life
5	Caucasian (U.S.)	c.182G>A p.(Gly61Glu) (pat)	Damaging by 5/5 (S, PP, MT, MA, F)	22/26024	0	Bilateral infantile glaucoma (<1 year of age) and iris hypoplasia	Father with mild iris dysplasia; paternal great grandfather with **adult-onset glaucoma**
		c.1064_1076del p.(Arg355Hisfs*69)	Premature truncation	NP	0		
6	Caucasian (Iran)	homozygous c.182G>A, p.(Gly61Glu) (mat + pat)	Damaging by 5/5 (S, PP, MT, MA, F)	22/26024	0	Bilateral CG and **sclerocornea**	Consanguinity; no history of ocular disorders
7	Caucasian (Lebanon)	**c.157G>A p.(Gly53Ser)** (mat)	Damaging by 5/5 (S, PP, MT, MA, F)	NP	0	Bilateral CG and Peters anomaly, right **microphthalmia**	Consanguinity; no history of ocular disorders
		c.1405C>T, p.(Arg469Trp) (pat)	Damaging by 4/5 (S, PP, MT, MA)	5/66734	0		

PCG=Primary congenital glaucoma; CG=Congenital Glaucoma; ASD=Anterior segment dysgenesis; NP=Not Present; (mat): mutation present in the mother, (pat): mutation present in the father; novel pathogenic alleles and phenotypic features are shown in **bold** font [a] Nucleotide numbering is relative to reference sequence NM_000104.3 where +1 is the A of the ATG initiation codon [b] Five prediction algorithms (SIFT (S), PolyPhen2 (PP), MutationTaster (MT), MutationAssessor (MA), FATHMM (F)) from dbNSFP 2.9 were accessed through SNP & Variation Suite (Golden Helix, Bozeman, MT) [c] Allele frequency for most closely related ethnic populations provided (European, S. Asian or Latino); in ExAC (Exome Aggregation Consortium; EXAC) [d] Number of homozygotes present in ExAC is noted Eu: European, SA: South Asian,

Figure 1. Photographs of patients. **A**: Photograph of Patient 1 with bilateral primary congenital glaucoma taken at 5 years of age. **B**: Photograph of Patient 4 with congenital glaucoma and anterior segment dysgenesis at 6 years of age.

one, p.(Tyr81Asn), was computed to be damaging by four of the five programs (Table 2). None of the cases carried a second mutation in *CYP1B1.* All four patients are Caucasian, the age of diagnosis ranged from 41 to 63 years of age, and all four patients were treated with medications to lower intraocular pressure (IOP) only (no surgical intervention). A family history of adult-onset glaucoma was noted in Patient 11, but no details were available.

Among the age- and ethnicity-matched controls without glaucoma, heterozygous previously reported pathogenic variants in *CYP1B1* associated with recessive PCG were identified in three control individuals, including the c.1103G>A; p.(Arg368His) variant in two and the c.1405C>T; p.(Arg469Trp) variant in one.

The previously reported rare c.685G>A, p.(Glu229Lys) variant and six common previously reported polymorphisms, g.3793T>C (c.-1–12C>T); c.142C>G, p.(Arg48Gly); c.355G>T, p.(Ala119Ser); c.1294G>C, p.(Leu432Val); c.1347T>C, p.(Asp449Asp); c.1358A>G, p.(Asn453Ser), were observed in the cases and controls at similar frequencies (Appendix 2).

DISCUSSION

Homozygous or compound heterozygous mutations in *CYP1B1* were identified in a high proportion of probands with PCG (2/8; 25%) and a significant portion of those with congenital glaucoma and ASD (3/22; 14%) or developmental phenotypes (2/11; 18%). The expansion of the *CYP1B1*

phenotypic spectrum to the more complex developmental ocular phenotypes of sclerocornea and microphthalmia seems to be consistent with the gene's predicted broad function. *Cyp1b1* demonstrates wide expression during development in animal models [47,48]. CYP1B1 was shown to be involved in retinoic acid (RA) synthesis [48,49], and functional analysis of variants associated with PCG showed abnormal metabolism of retinol by mutant proteins [37]. Mutations of other genes in the retinoic acid pathway are known to cause isolated and syndromic anophthalmia or microphthalmia [50]. Therefore, expanded ocular phenotypes associated with mutations in *CYP1B1* are not surprising. At the same time, as these additional anomalies occurred in consanguineous pedigrees, it is possible that other inherited recessive variants contributed to these phenotypes.

Most of the identified mutations in the pediatric cohort have been previously associated with congenital glaucoma and/or ASD. The two novel mutations detected in Patient 2 (c.1325delC, p.(Pro442Glnfs*15)) and Patient 7 (c.157G>A, p.(Gly53Ser)) show strong evidence for pathogenicity. In terms of recurrent mutations, the c.182G>A, p.(Gly61Glu) allele exhibits a possible association with ASD and other ocular phenotypes as this variant was present in two probands with ASD or sclerocornea reported here, as well as in 11 previously published cases with Peters anomaly (five), ectropion uveae with partial aniridia (five), and/or acquired peripheral iris degeneration (one) [12,15,51].
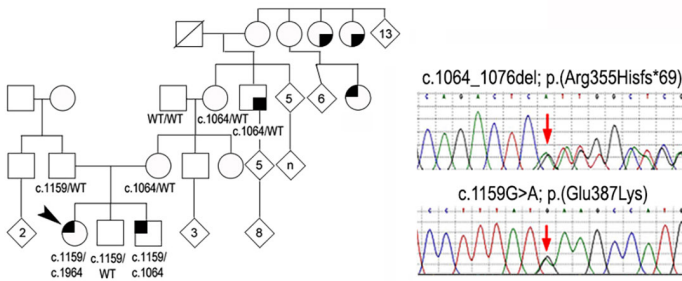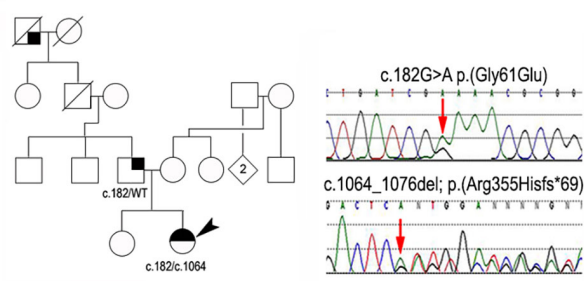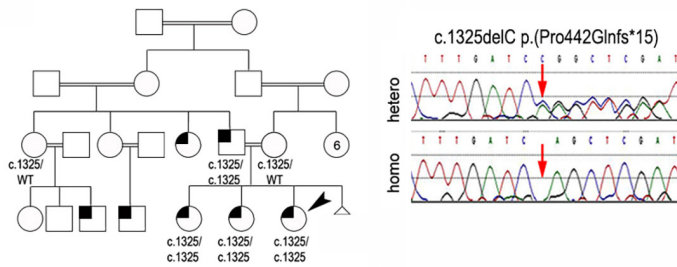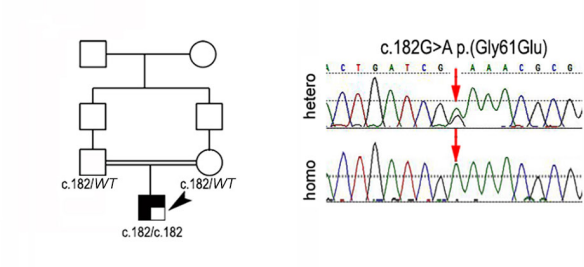
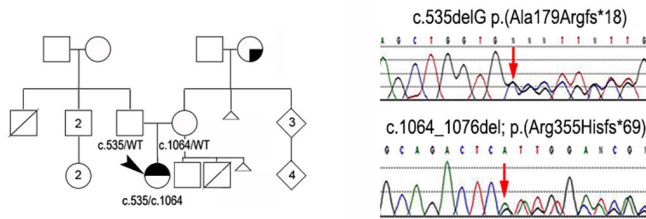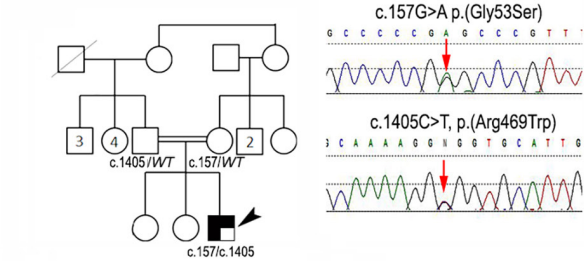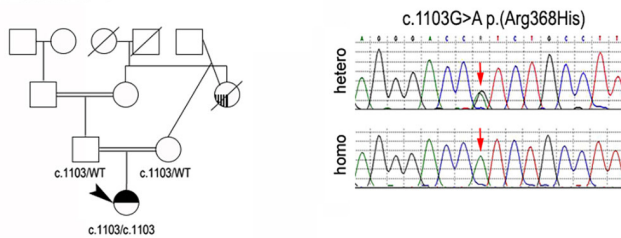Figure 2. Pedigrees and *CYP1B1* mutation information. Patients 1–7 are indicated with black arrowheads in each pedigree (**A-G**). DNA sequencing chromatograms of *CYP1B1* pathogenic alleles are shown, and variant positions are indicated with red arrows.

The contribution of *CYP1B1* to POAG is less clear. Although variants in *CYP1B1* were not found to make a significant contribution to POAG in the unselected cohort screened here, heterozygous loss-of-function variants appear likely to increase the risk of POAG. First, in this report, multiple *CYP1B1* pedigrees with congenital phenotypes demonstrated adult-onset glaucoma in heterozygous (or presumed heterozygous) relatives of probands with congenital glaucoma, a novel and interesting finding. A heterozygous loss-of-function allele, c.1064_1076del, p.(Arg355Hisfs*69), was identified in the unselected POAG population and in two families with POAG in relatives of a proband affected with congenital glaucoma. The same heterozygous frameshift mutation was also reported in two French unrelated POAG probands [21], a different heterozygous frameshift mutation (c.1311–1315del5) was reported in one Indian patient with JOAG [23], and two other heterozygous nonsense mutations (c.171G>A, p.Trp59* and c.1084C>T, p.Gln362*) were

reported in three other families with JOAG/POAG; family history was noted for one family, in which a sibling with pseudoexfoliative glaucoma carried the same truncating mutation [24,52,53]. None of these truncating mutations were observed in age-matched non-glaucoma controls.

Although two recent meta-analyses found no association between *CYP1B1* polymorphisms and POAG [54,55], these studies analyzed common variants with allele frequencies between 15% and 62% and thousands of homozygotes present in the general population, not rare loss-of-function alleles as identified in this study. Previous functional analyses of CYP1B1 mutant proteins suggested that variants that affect the metabolism of 17β-estradiol may contribute to POAG through *MYOC* overexpression when 17β-estradiol metabolism is decreased or through increased levels of reactive oxygen species and apoptosis when metabolism is increased [36,37]. For many of these variants, the decreased enzyme activity was shown to correlate with decreased protein stability leading to decreased levels of protein; consistent with this, the frameshift mutation would be expected to result in decreased levels of functional CYP1B1 protein. Given the rarity of loss-of-function variants (as demonstrated by the rarity of PCG in the general population), it is perhaps not surprising that loss-of-function variants in *CYP1B1* would explain only a small proportion of the much more common POAG. Although many factors, both genetic and environmental, are involved in POAG pathogenesis, a heterozygous loss-of-function variant in *CYP1B1*, particularly one that affects the metabolism of 17β-estradiol seems likely to confer an increased risk of glaucoma in adulthood. Further study and characterization of missense alleles is needed to determine which missense variants affect CYP1B1 function (and thus may confer an increased risk of glaucoma) and which are benign variants.

In summary, *CYP1B1* represents a major gene for primary congenital glaucoma and a significant contributor to congenital glaucoma associated with ASD. In addition, the phenotype associated with mutations in *CYP1B1* has been expanded to include sclerocornea and microphthalmia, but the gene does not appear to be involved in developmental ocular phenotypes that do not include congenital glaucoma. Although *CYP1B1* does not appear to be a major contributor to POAG in the general population, the presence of the same heterozygous frameshift variant, c.1064_1076del, p.(Arg355Hisfs*69), in an unselected POAG proband and two CG pedigrees with a family history of POAG, as well as the identification of POAG in a relative of a third CG proband, suggests that some *CYP1B1* pathogenic variants increase the risk for POAG. Until more is known about which alleles are associated with increased risk, heterozygous carrier relatives

TABLE 2. SUMMARY OF *CYP1B1* VARIANTS IN POAG CASES AND AGE-MATCHED CONTROLS.

Case	*CYP1B1* mutation[a]	Functional prediction[b]	Allele frequency[c]	#homozygote controls[d]	POAG	Age[e]	Cup/disc ratio	Family history
Patient 8	c.1064_1076del p.(Arg355Hisfs*69)	Premature truncation	NP	0	Yes	47 years	0.6/0.7	None
Patient 9	c.241T>A p.(Tyr81Asn)	Damaging by 4/5 (S, PP, MT, MA)	159/22812	1 Eu, 2 SA, 1 Fn	Yes	41 years	0.4/0.6	None
Patient 10	c.1328C>G p.(Arg443Gly)	Benign by 5/5	86/66736	14 Af	Yes	63 years	0.85/ 0.875	Unknown
Patient 11	c.35C>T (p.Pro12Leu)	Benign by 5/5	4/62318	1 Af	Yes	51 years	0.4/0.75	Positive, details unknown
Control 1	c.1103G>A (p.Arg368His)	Damaging by 4/5 (S, PP, MT, MA)	184/63292	9 SA, 1 Eu	No	57 years	-	-
Control 2	c.1103G>A (p.Arg368His)	Damaging by 4/5 (S, PP, MT, MA)	184/63292	9 SA, 1 Eu	No	60 years	-	-
Control 3	c.1405C>T (p.Arg469Trp)	Damaging by 4/5 (S, PP, MT, MA)	5/66734	0	No	71 years	-	-

POAG: Primary open angle glaucoma; NP=Not Present; hom- homozygotes; [a] Nucleotide numbering is relative to reference sequence NM_000104.3 where +1 is the A of the ATG initiation codon [b] Five prediction algorithms (SIFT (S), PolyPhen2 (PP), MutationTaster (MT), MutationAssessor (MA), FATHMM (F)) from dbNSFP 2.9 were accessed through SNP & Variation Suite (Golden Helix, Bozeman, MT) [c] Allele frequency for Caucasian populations in ExAC (Exome Aggregation Consortium; EXAC) [d] Number of homozygotes present in ExAC is noted; Af: African, Eu: European, Fn: Finnish, SA: South Asian [e] Age at diagnosis (for Patients 8-11) or at last normal eye exam (for Controls 1-3)

of patients with pediatric *CYP1B1* phenotypes should be monitored for adult-onset glaucoma.

APPENDIX 1.

Summary of heterozygous *CYP1B1* variants of uncertain significance and patient features. To access the data, click or select the words "Appendix 1."

APPENDIX 2.

Summary of *CYP1B1* polymorphisms in PMRP POAG cases and non-POAG controls. To access the data, click or select the words "Appendix 2."

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