• Clinical Research •

Correlation between pathogenic *CYP1B1* variants and trabeculodysgenesis under ultrasound biomicroscopy in primary congenital glaucoma

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Received: 2025-04-25 Accepted: 2025-08-15

Abstract

● **AIM:** To explore the genetic variants of trabeculodysgenesis under ultrasound biomicroscopy (UBM) and its association with surgical outcomes in patients with primary congenital glaucoma (PCG).

● **METHODS:** In this prospective case series, consecutive patients with PCG underwent microcatheter-assisted trabeculotomy (MAT) and followed for at least 24-month after surgery. All participants underwent UBM and whole-exome sequencing prior to MAT and were classified into two groups with severe trabeculodysgenesis or mild trabeculodysgenesis under UBM. Surgical success was defined as a postoperative IOP of ≤21 mm Hg with at least a 20% reduction from preoperative IOP without additional medical or surgical therapy.

● **RESULTS:** Severe trabeculodysgenesis was observed in 23 (40%) eyes of 14 patients (median age: 57mo, range: 3-169mo; 11 males) with 8 carrying causative *CYP1B1* variants, while mild trabeculodysgenesis occurred in 34 eyes (60%) of 18 patients (median age: 23.5mo, range: 3-110mo; 12 males) without any causative variants. The success rate of MAT was 0 in patients with causative *CYP1B1* gene variants and 75.0% in those without (P<0.001). Cox regression survival analysis showed that carrying *CYP1B1* gene variants [OR_{CYP1B1}=0.356 (95%CI: 0.132, 0.962), P=0.042] and having severe trabeculodysgenesis [OR_{Type}=0.116 (95%CI: 0.034, 0.403), P=0.001] were associated with a higher risk of surgical failure.

● **CONCLUSION:** PCG patients with severe trabeculodysgenesis under UBM are prone to harbor causative *CYP1B1* gene variants, which could serve as a valuable predictor of potential Schlemm's canal dysgenesis and MAT prognosis. Genetic screening in patients with severe trabeculodysgenesis under UBM is beneficial for genetic counseling and may help reduce the incidence of complex cases.

● **KEYWORDS:** primary congenital glaucoma; *CYP1B1* gene variants; microcatheter-assisted trabeculotomy
DOI:10.18240/ijo.2026.06.08

Citation: Gao Y, Lin DT, Zhou T, He LH, Zhao KX, Yu XW, Deng L, Fan ZG, Shi Y. Correlation between pathogenic *CYP1B1* variants and trabeculodysgenesis under ultrasound biomicroscopy in primary congenital glaucoma. *Int J Ophthalmol* 2026;19(6):1079-1087

INTRODUCTION

Primary congenital glaucoma (PCG) is a potentially blinding disease caused by developmental anomalies in aqueous humor outflow structures, leading to elevated intraocular pressure (IOP) in children under three[1]. PCG prevalence varies from 1 in 10 000 in Western populations to 1 in 1250 among Slovak Roma and accounts for 2%–15% of childhood blindness cases[2-3]. Cytochrome P450 family 1 subfamily B member 1 (*CYP1B1*, OMIM 601771) is the most common identifiable cause of PCG worldwide, potentially halting the normal development in the trabecular meshwork (TM) and outflow pathways[4]. Other implicated genes include latent transforming growth factor-beta binding protein 2 (*LTBP2*), forkhead box C1 (*FOXC1*), heterozygous alleles in the angiopoietin receptor-encoding gene (TEK receptor tyrosine kinase, *TEK*) and angiopoietin 1 (*ANGPT1*)[5]. *CYP1B1* mutations exhibit significant ethnic variability, ranging from

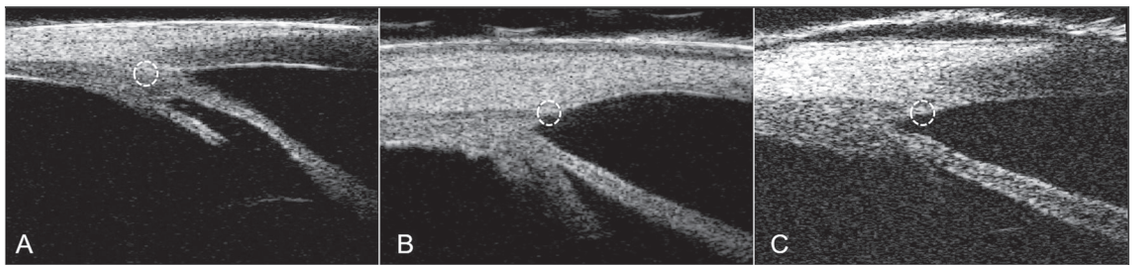

Figure 1 Types of trabeculodysgenesis under 80-MHz ultrasound biomicroscopy A: Type 1 trabeculodysgenesis. Severe type corresponding to insertion of the ciliary processes or iris before the scleral spur, overlapping of the posterior portion of the TM by uveal tissue, and an absent or blunt anterior chamber recess; B: Type 2 trabeculodysgenesis. Mild type with normal roots of iris and ciliary processes comparable to those in patients without PCG; C: Anterior chamber angle of an unaffected contralateral eye in a patient with unilateral PCG. The scleral spur is highlighted by a white dashed circle in each image. TM: Trabecular meshwork; PCG: Primary congenital glaucoma.

20% in Japanese to 100% in Saudi Arabians and Slovak Roma[6-7]. In the Chinese Han population, 17.2% of PCG cases involve *CYP1B1* mutations[8]. While genetic screening for *CYP1B1* variants is not yet a standard diagnostic tool, studies suggest certain pathogenic variants correlate with more severe phenotypes and poorer prognoses[9-11]. This suggests that screening for these variants could aid in counseling and follow-up strategies.

Microcatheter-assisted trabeculotomy (MAT) is more effective than traditional angle surgeries for PCG, offering precise Schlemm's canal (SC) localization and angle opening with a single incision[11-16]. Our previous study found that PCG patients with severe trabeculodysgenesis, as assessed by ultrasound biomicroscopy (UBM), had lower MAT success rates[16]. Since previous surgical failures can complicate outcomes and heighten the risk of blindness[17], identifying cases at high risk for MAT would be highly beneficial. Moreover, we speculated that severe trabeculodysgenesis may indicate high-risk genetic variants, offering insights into genotype-phenotype correlations. In this study, we explored genetic variants in Chinese PCG patients with varying degrees of trabeculodysgenesis and their correlation with MAT outcomes, aiming to improve PCG management and reduce blindness.

PARTICIPANTS AND METHODS

Ethical Approval This study adhered to the Declaration of Helsinki and was prospectively approved by the Ethics Committee of Beijing Tongren Eye Center. Each patient's legal guardian or representative signed an informed consent. The clinical trial was registered under the Chinese Clinical Trials Registry (ChiCTR-OCC-15005789).

Participants This prospective study included consecutive PCG patients who underwent MAT with an illuminated microcatheter (iTRACK 250A; iScience Interventional, Menlo Park, CA, USA) at Beijing Tongren Eye Center from July 2021 to July 2023. The diagnosis of PCG was based on the presence of at least two of the following clinical features: 1) increased corneal diameter (>12 mm) along with elevated IOP (>21 mm Hg), 2) Haab striae, 3) corneal edema, 4) increased cup-to-disc ratio. Exclusion criteria included other ocular or systemic anomalies, prior surgery affecting UBM assessment, or follow-up less than 2y. Both eyes were analyzed if eligible.

Clinical Characteristic Evaluation Clinical data collected included gender, affected eye type (unilateral/bilateral), age at onset and surgery, IOP, medication count, corneal diameter and opacity score. IOP was measured preoperatively and at 1, 3, 6, 9, 12, 18, and 24mo postoperatively using the Icare tonometer (Icare TA01i; Icare Finland Oy, Espoo, Finland) under chloral hydrate sedation (median dose: 75 mg/kg). Age at onset was classified as neonatal (0–1mo), infantile (>1–24mo), or late-onset (>2y)[18-19]. The grading criteria of corneal opacity were as follows: 1+, complete corneal transparency; 2+, mild corneal haze with visible iris; 3+, severe opacity obscuring the iris; 4+, severe corneal opacity with neovascularization. Additionally, 0.5 was added to the corneal opacity score when Haab striae were noted.

Assessment of Trabeculodysgenesis Prior to surgery, the anterior chamber angle was examined using the iUltrasound imaging system (iScience Interventional, Inc, Menlo Park, California, USA) in the supine position under general anesthesia. A low-viscosity gel was applied before placing the self-contained probe over the eye at the 3, 6, 9, and 12 o'clock meridians, capturing at least 20 images per position. The image processing and assessment for trabeculodysgenesis have been described previously[16]. Briefly, two investigators (Shi Y & Lin DT), who had previously calibrated their understanding of anatomical landmarks using 20 sample images, assessed the patients' scan images. After identifying the scleral spur (the joint point of the inner surface of the TM and sclera), the recruited eyes were grouped into two types: Type 1, severe trabeculodysgenesis, corresponded to those with insertion of ciliary processes and/or iris before the scleral spur; Type 2, mild trabeculodysgenesis, had normal roots of iris and ciliary processes (Figure 1).

Variant Analysis Extensive family histories were collected for each patient to create a pedigree and to perform whole-exome sequencing (WES) to identify disease-associated genes in all probands and their available relatives. Genomic DNA was extracted from peripheral blood and the coding DNA was enriched using the SureSelect Human All Exon Kit (Agilent V6, Santa Clara, CA, USA). Paired-end sequencing was performed with the Novaseq 6000 sequencer (Illumina, San Diego, CA, USA) with an average sequencing depth of 102.53X. Alignment and variant calling were performed according to the Genome Analysis Toolkit (GATK) and the Variant Call Format (VCF) files were then analyzed using ANNOVAR software. The variants were filtered using the following criteria: 1) low-quality variants were removed by GATK recommended filters; 2) variants present in the public genetic variant databases, Genome Aggregation Database (GnomAD v2.0.1), with an allele frequency <2% were included; 3) nonsense variants, frameshift variants, splice site, or predicted damaging missense variants by Combined Annotation Dependent Depletion (CADD), Sorting Intolerant From Tolerant (SIFT), PolyPhen2, or VariantTaster were included. Additionally, the American College of Medical Genetics and Genomics (ACMG) guidelines were employed. The sequencing depth in subjects in the discovery stage ranged from 99.17 to 259.84 and the genome coverage ranged from 99.30% to 99.88%. Patients were further considered to have causative variants when the variants co-segregated with PCG within the family and met the following criteria based on published data: 1) One variant was present in a gene with autosomal dominant (AD) or X-linked (XL) inheritance; 2) two heterozygous variants or one homozygous variant were present in a gene with autosomal recessive (AR) inheritance. Otherwise, patients were identified as having no causative variants.

Surgical Procedure and Criteria for Surgical Success A single experienced surgeon (Shi Y) performed all procedures using a standardized approach[16]. After locating SC, a microcatheter was advanced under direct visualization. If successful, 360-degree trabeculotomy was achieved by retrieving the catheter and pulling both ends. If catheterization was incomplete due to obstruction or misdirection, an alternate entry from the other end was attempted. For cases where the catheter advanced at least 180 degrees, a scleral cut down was performed to grasp the catheter tip and complete partial trabeculotomy. If advancement was under 180 degrees, a partial *ab externo* trabeculotomy was performed using a Harms trabeculotome. The scleral flaps and peritomy were closed with sutures, and chamber irrigation was conducted if needed. Postoperatively, patients received tobramycin-dexamethasone and pranoprofen drops for 2-4wk, along with pilocarpine 2% for 3mo to prevent synechiae. Surgical success was defined as a postoperative IOP ≤21 mm Hg with at least a 20% reduction from baseline, without additional medical or surgical intervention beyond pilocarpine.

Statistical Analysis Statistical analysis was conducted using SPSS (V.23.0; SPSS, Chicago, IL, USA) with a significance level of $P<0.05$. Categorical data were counted by frequency and analyzed using Chi-square or Fisher's exact tests. Non-normally distributed quantitative data were expressed as medians (range) and analyzed by Mann-Whitney *U* test. A linear mixed model adjusted for correlation between both eyes in bilateral cases was employed to compare differences between eyes of two groups. Kaplan-Meier analysis evaluated surgical success, with survival curves compared using log-rank and Chi-square tests. Cox regression was used to assess factors influencing success rates, including age at onset and surgery, gender, preoperative IOP, medications, corneal opacity, corneal diameter, trabeculodysgenesis type, SC catheterization extent, and presence of causative *CYP1B1* gene variants.

RESULTS

Subject Characteristics A total of 32 patients from 29 unrelated families were enrolled, including 23 males (72%) and 9 females (28%). The ages of patients at the time of UBM examination and surgery ranged from 3 to 169mo, with a median age of 31mo. Bilateral involvement was observed in 30 patients (94%), while 2 patients (6%) had unilateral involvement. After excluding 5 eyes due to prior angle surgeries, 57 PCG eyes remained for analysis. Each affected eye consistently showed the same type of trabeculodysgenesis across all four quadrants. Additionally, all patients with bilateral involvement had matching trabeculodysgenesis types in both eyes. Twenty-three eyes (40%) in 14 patients exhibited severe trabeculodysgenesis, while 34 eyes (60%) in 18 patients had mild trabeculodysgenesis (Table 1).

Comparison of Clinical Characteristics Between Patients with Different Trabeculodysgenesis Types Table 1 shows a comparison of clinical characteristics between patients with different types of trabeculodysgenesis. Patients with severe trabeculodysgenesis had an earlier stage of onset-age than those with mild trabeculodysgenesis ($P=0.009$), but no significant differences were found in gender, eye laterality, age at surgery, preoperative IOP, medications, corneal opacity, or diameter (all $P>0.05$). Severe cases had significantly less SC catheterization ($P<0.001$), with 70% requiring partial trabeculotomy using a Harms trabeculotome, compared to 3% in the mild group.

Analysis of Gene Variants and Genotype-Phenotype Correlations Among 32 patients, pathogenic variants were identified in 16 (50%) from 14 families, while 16 patients (50%) from 10 families had no variants. Fourteen *CYP1B1*

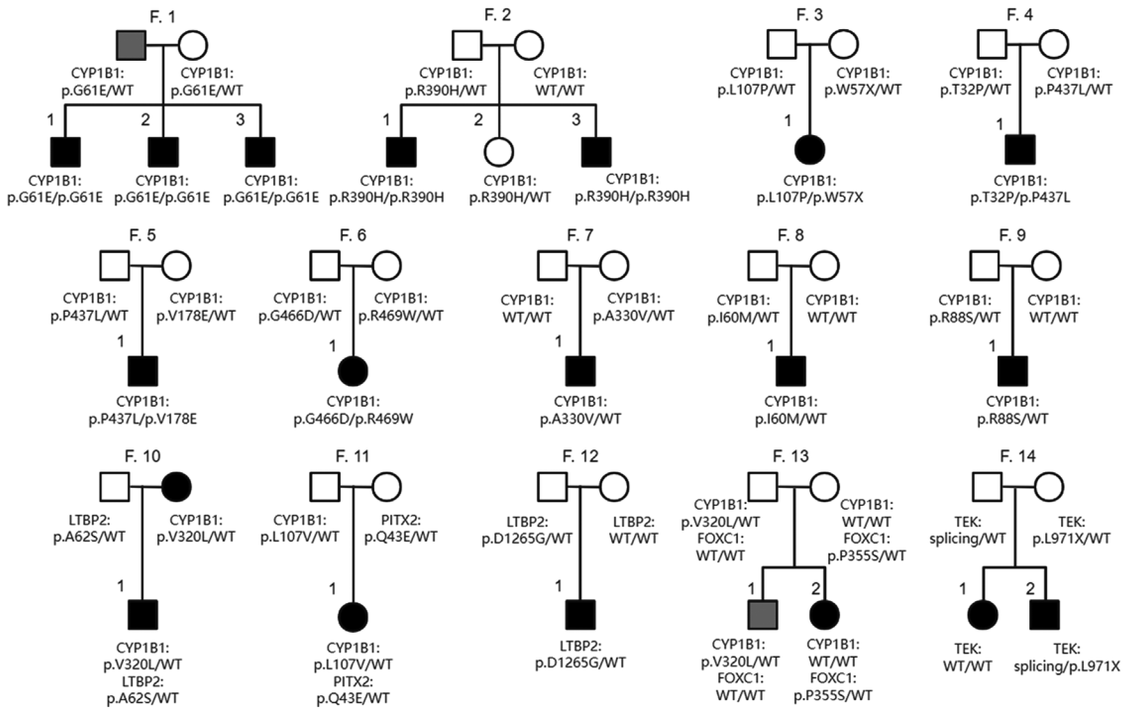

Figure 2 Families of PCG patients with related gene variants Pedigrees of 14 PCG families with PCG-related variants including *CYP1B1, LTBP2, PITX2, FOXC1 and TEK*. Filled and empty symbols represent affected and unaffected individuals, grey symbols represent JOAG, respectively. Genotypes are written at the bottom of the enrolled individuals. PCG: Primary congenital glaucoma; JOAG: Juvenile open angle glaucoma; *CYP1B1*: Cytochrome P450 family 1 subfamily B member; *LTBP2*: latent transforming growth factor-beta binding protein 2; *PITX2*: Paired like homeodomain transcription factor 2; *FOXC1*: Forkhead box C1; *TEK*: TEK receptor tyrosine kinase.

Table 1 Comparison of clinical characteristics and *CYP1B1* variants between patients with different types of trabeculodysgenesis and different status of carrying causative variants

Parameters	Severe trabeculodysgenesis	Mild trabeculodysgenesis	*P*	Causative variants	No causative variants	*P*
No. of patients	14	18	0.480	8	24	0.005[b]
No. of eyes	23	34	0.792	13	44	<0.001[c]
Type of affected eyes (unilateral/bilateral)	0/14	2/16	0.492	0/8	2/22	1.000
Gender (male/female)	11/3	12/6	0.694	6/2	17/7	1.000
Recruited eye (OD/OS)	10/13	16/18	1.000	5/8	21/23	0.753
Stage of onset-age (stage1/2/3)[a]	13/6/4	6/16/12	0.009	10/3/0	9/19/16	<0.001[c]
Age at operation [mo, median (range)]	57 (3–169)	23.5 (3–110)	0.059	23 (5–169)	33.5 (3–110)	0.675
Preoperative IOP [mm Hg, median (range)]	33.5 (23–51)	32 (20–57)	0.662	32 (24–44)	32 (20–57)	0.631
Preoperative number of medication [median (range)]	3 (2–3)	2 (0–4)	0.064	3 (2–3)	2.5 (0–4)	0.066
Preoperative corneal diameter [mm, median (range)]	13.0 (10.0–16.0)	13.0 (11.0–14.0)	0.903	13.0 (11.5–15.0)	13.0 (10.0–16.0)	0.877
Corneal opacity score [median (range)]	2.0 (1.0–4.0)	1.5 (0.5–3.5)	0.261	2.0 (1.0–4.0)	1.5 (0.5–3.5)	0.217
Extent of SC catheterization (360-degree/≥180-degree/<180-degree)	1/6/16	29/4/1	<0.001[c]	0/4/9	30/6/8	<0.001[c]
Trabeculodysgenesis (type 1/type 2)	-	-	-	13/0	10/34	<0.001[c]
Percentage of patients with causative variant, *n* (%)	8 (57.1)	0	<0.001[c]	-	-	-
Percentage of patients with causative variants of *CYP1B1*, *n* (%)	8 (57.1)	0	<0.001[c]	-	-	-
Percentage of patients with at least one variant of *CYP1B1*, *n* (%)	10 (71.4)	3 (16.7)	0.003[b]	8 (100)	5 (20.8)	<0.001[c]

[a]Stage of onset-age was divided according to the classification of primary congenital glaucoma: 1: Neonatal or newborn onset (0–1mo); 2: Infantile onset (>1–24mo); 3: Late onset or late-recognized (>2y). [b]*P*<0.01, [c]*P*<0.001. IOP: Intraocular pressure; SC: Schlemm's canal; *CYP1B1*: Cytochrome P450 family 1 subfamily B member 1; OD: Right eye; OS: Left eye.

missense variants were found, including three novel ones (p.Leu107Pro, p.Thr32Pro, and p.Ala330Val)[8,20-25] (Figure 2, Table 2). Only eight patients with homozygous or compound heterozygous *CYP1B1* variants were considered having causative variants and genetically diagnosed[26]. Among them, 3 patients with *CYP1B1* homozygous variants (p.Gly61Glu) were from one family with consanguineous parents, and their father, carrying a heterozygous variant (p.Gly61Glu), had juvenile open angle glaucoma (JOAG). The remaining 5 patients were unrelated cases with non-consanguineous parents.

Table 2 Analysis of variants in 16 primary congenital glaucoma patients and their clinical outcomes

Case ID	Gender	Gene	Zygosity	Inheritance	Type of variant	AA change	cDNA change	SIFT	PolyPhen_2	MutationTaster	CADD PHRED	REVEL score	ΔSpliceAI score	ACMG classification	Type of trabeculodysgenesis	Extent of SC catheterization	Surgical outcome[a]	Reference
F1-1	Male	*CYP1B1*	Hom	AR	Missense	p.Gly61Glu	c.182G>A	-	D	A	23.6	0.795	-	LP	1	OU: 3	OU: F	26
F1-2	Male	*CYP1B1*	Hom	AR	Missense	p.Gly61Glu	c.182G>A	-	D	A	23.6	0.795	-	LP	1	OD: 2 OS: 3	OU: F	26
F1-3	Male	*CYP1B1*	Hom	AR	Missense	p.Gly61Glu	c.182G>A	-	D	A	23.6	0.795	-	LP	1	OS: 2	OS: F	26
F2-3	Male	*CYP1B1*	Hom	AR	Missense	p.Arg390His	c.1169G>A	-	D	D	32	0.982	-	LP	1	OS: 3	OS: F	27
F3-1	Female	*CYP1B1*	Het	AR	Missense	p.Leu107Pro	c.320T>C	-	D	D	25.1	0.802	-	VUS	1	OU: 3	OU: F	Novel
		CYP1B1	Het	AR	Nonsense	p.Trp57Ter	c.171G>A	-	-	A	37	-	-	P				28
F4-1	Male	*CYP1B1*	Het	AR	Missense	p.Pro437Leu	c.1310C>T	-	D	D	28.1	0.924	-	LP	1	OU: 2	OU: F	27
		CYP1B1	Het	AR	Missense	p.Thr32Pro	c.94A>C	-	B	N	9.336	0.213	-	VUS				Novel
F5-1	Male	*CYP1B1*	Het	AR	Missense	p.Pro437Leu	c.1310C>T	-	D	D	28.1	0.924	-	LP	1	OS: 3	OS: F	27
		CYP1B1	Het	AR	Missense	p.Val178Glu	c.533T>A	-	D	D	25.6	0.821	-	LP				29
F6-1	Female	*CYP1B1*	Het	AR	Missense	p.Gly466Asp	c.1397G>A	-	D	D	27	0.954	-	P	1	OU: 3	OU: F	30
		CYP1B1	Het	AR	Missense	p.Arg469Trp	c.1405C>T	-	D	A	25.9	0.723	-	LP				31
F7-1	Male	*CYP1B1*	Het	AR	Missense	p.Ala330Val	c.989C>T	-	D	D	32	0.94	-	VUS	1	OU: 3	OU: F	Novel
F8-1	Male	*CYP1B1*	Het	AR	Missense	p.Ile60Met	c.180C>G	-	D	D	23.1	0.535	-	LP	2	OU: 1	OU: S	32
F9-1	Male	*CYP1B1*	Het	AR	Missense	p.Arg88Ser	c.262C>A	-	P	D	24.3	0.587	-	VUS	2	OD: 1	OD: S	Novel
F10-1	Male	*CYP1B1*	Het	AR	Missense	p.Val320Leu	c.958G>T	-	P	D	23.9	0.349	-	VUS	2	OD: 2	OU: S	33
		LTBP2	Het	AR	Missense	p.Ala62Ser	c.184G>T	T	B	N	17.7	0.082	-	VUS				Novel
F11-1	Female	*CYP1B1*	Het	AR	Missense	p.Leu107Val	c.319C>G	-	D	D	23.2	0.647	-	VUS	1	OD: 2	OD: S	32
		PITX2	Het	AD	Missense	p.Gln43Glu	c.127C>G	T	B	D	22.9	0.324	-	VUS				Novel
F12-1	Male	*LTBP2*	Het	AR	Missense	p.Asp1265Gly	c.3794A>G	T	B	D	16.09	0.281	-	VUS	2	OD: 1	OD: S; OS: F	Novel
F13-2	Female	*FOXC1*	Het	AD	Missense	p.Pro355Ser	c.1063C>T	T	P	D	20.6	0.297	-	VUS	2	OD: 1; OS: 2	OD: S, OS: F	Novel
F14-2	Male	*TEK*	Het	AD	Nonsense	p.Leu971Ter	c.2912T>A	-	-	A	48	-	-	LP	2	OD: 1; OS: 2	OU: F	Novel
		TEK	Het	AD	Splicing	N/A	c.3300+4A>C	-	-	-		0.795	0.15	VUS				Novel

F: Family; *CYP1B1*: Cytochrome P450 family 1 subfamily B member; *LTBP2*: latent transforming growth factor-beta binding protein 2; *PITX2*: Paired like homeodomain transcription factor 2; *FOXC1*: Forkhead box C1; *TEK*: TEK receptor tyrosine kinase; Het: Heterozygous; Hom: Homozygous; AD: Autosomal dominant; AR: Autosomal recessive; AA: Amino acids; N/A: Not available; SIFT: Sorting intolerant from tolerant; CADD PHRED: Combined annotation dependent depletion PHRED-like scaled score; REVEL score: Rare exome variant ensemble learner score; ACMG: American College of Medical Genetics and Genomics guidelines; SC: Schlemm's canal. SIFT: T: Tolerated. PolyPhen-2: B: Benign; D: Probably damaging; P: Possibly damaging. MutationTaster: A: Disease causing automatic; D: Disease causing; N: Polymorphism. CADD PHRED threshold for deleteriousness is >15. REVEL threshold for deleteriousness is >0.659. SpliceAI threshold for deleteriousness is >0.2. ACMG classification: P: Pathogenic; LP: Likely pathogenic; VUS: Variants of uncertain significance (insufficient or contradictory evidence prevents definitive classification of the variant as benign or pathogenic). Type of trabeculodysgenesis: 1: Severe trabeculodysgenesis; 2: Mild trabeculodysgenesis. [a]Surgical outcome after microcatheter-assisted trabeculotomy: OD: Right eye; OS: Left eye; OU: Both eyes; S: Success; F: Failure.

Int J Ophthalmol, Vol. 19, No. 6, Jun. 18, 2026 www.ijo.cn
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Table 3 Postoperative IOP and number of medications between patients with severe and mild trabeculodysgenesis

median (range)

Follow-up	Postoperative IOP			Number of postoperative medications		
	Severe	Mild	*P*	Severe	Mild	*P*
1mo	22 (7, 46)	14 (10, 25)	0.014[a]	0.5 (0, 4)	0 (0, 1)	<0.001[c]
6mo	20 (10, 33)	13 (9, 30)	0.006[b]	2 (0, 4)	0 (0, 2)	<0.001[c]
9mo	22.5 (10, 32)	14 (8, 33)	<0.001[c]	2 (0, 4)	0 (0, 2)	<0.001[c]
12mo	19 (10, 31)	14 (9, 22)	0.001[c]	2 (0, 4)	0 (0, 1)	<0.001[c]
18mo	21 (11, 31)	14 (8, 27)	0.001[c]	2 (0, 4)	0 (0, 3)	<0.001[c]
24mo	18 (10, 34)	14 (8, 27)	0.004[b]	3 (0, 4)	0 (0, 2)	<0.001[c]

[a]*P*<0.05, [b]*P*<0.01, [c]*P*<0.001. IOP: Intraocular pressure.

Other genetic variants including *LTBP2*, paired like homeodomain transcription factor 2 (*PITX2*), *FOXC1*, and *TEK* were found in five patients (16%), two of whom also carried a heterozygous *CYP1B1* variant. However, these patients, along with three others who had only a heterozygous *CYP1B1* variant, were not considered to carry causative variants, as they did not follow co-segregate inheritance patterns.

All patients with causative *CYP1B1* gene variants exhibited severe trabeculodysgenesis and had earlier onset, less SC catheterization than those without (all *P*<0.001). Notably, no patient with causative *CYP1B1* gene variants achieved 360-degree SC catheterization (Table 1). Additionally, two other patients in the severe trabeculodysgenesis group carried a *CYP1B1* variant each: one had a heterozygous *CYP1B1* variant (p.Ala330Val), and the other had a heterozygous *CYP1B1* variant (p.Leu107Val) along with a *PITX2* variant (p.Gln43Glu). Four patients with severe trabeculodysgenesis showed no pathogenic variants. In the mild trabeculodysgenesis group, two patients had a heterozygous *CYP1B1* variant (p.Ile60Met and p.Arg88Ser), one patient had a heterozygous *CYP1B1* variant (p.Val320Leu) combined with a *LTBP2* variant (p.Ala62Ser), and individual variants *LTBP2* (p.Asp1265Gly) and *FOXC1* (p.Pro355Ser) were found in the other 2 patients. One patient had compound heterozygous *TEK* variants (p.Leu971Ter/splicing), while 12 patients showed no pathogenic variants. Table 2 provides a detailed list of gene variants for both groups.

Surgical Outcomes Between Patients with Different Trabeculodysgenesis Types All 57 enrolled eyes underwent MAT surgery and were followed for 24mo. Due to uncontrolled IOPs, five eyes (22%) in the severe group and two eyes (6%) in the mild group required trabeculectomy and transscleral cyclophotocoagulation at 9, 12, or 24mo post-surgery. These cases were classified as surgical failures, and their follow-ups were discontinued. A linear mixed model with time as a repeated measure showed a significant reduction in IOP and the number of medications from baseline at all postoperative visits for both groups (both *P*<0.05). However, the severe group consistently had higher postoperative IOPs and required more medications than the mild group at all postoperative visits (all *P*<0.05; Table 3). Cumulative surgical success rates at various postoperative points are shown in Figure 3A. At 24mo, the success rates were 13.6% for the severe group and 88.2% for the mild group [*P*<0.001; Kaplan-Meier life table and log-rank (Mantel-Cox) analysis; Figure 3A].

Surgical Outcomes Among Patients with Different Genotypes Among the 13 eyes from 8 patients with causative *CYP1B1* gene variants, none achieved successful surgical outcomes. Of the 5 patients with heterozygous *CYP1B1* gene variants, only 1 patient with severe trabeculodysgenesis experienced surgical failure in both eyes. One eye with severe trabeculodysgenesis and five eyes with mild trabeculodysgenesis maintained controlled IOP over the two-year follow-up. Additionally, one patient with mild trabeculodysgenesis and compound heterozygous *TEK* variants underwent 360-degree trabeculotomy in both eyes, which ultimately failed to control IOP. Two patients with mild trabeculodysgenesis, each carrying a heterozygous variant in *LTBP2* or *FOXC1,* had one eye with a successful outcome and one with a failure (Table 2). Kaplan-Meier survival analysis [log-rank (Mantel-Cox) test] indicated a 0 success rate in patients with causative *CYP1B1* gene variants, while those without causative gene variants had a 75.0% success rate (*P*<0.001; Figure 3B) at the 24-month follow-up.

Cox regression survival analysis (stepwise regression) of the 57 eyes revealed that carrying causative *CYP1B1* [OR_{CYP1B1}=0.356 (95%CI: 0.132, 0.962; *P*=0.042)] and having severe trabeculodysgenesis [OR_{Type}=0.116 (95%CI: 0.034, 0.403, *P*=0.001)] were associated with a higher risk of surgical failure.

The risk function was defined as:

$$\frac{h_t}{h0_{(t)}} = e^{(-1.032\times CYP1B1-2.151\times type)}$$

In this equation, *h0(t)* represents the amount of background risk. *CYP1B1* is the gene status of patients (those carrying

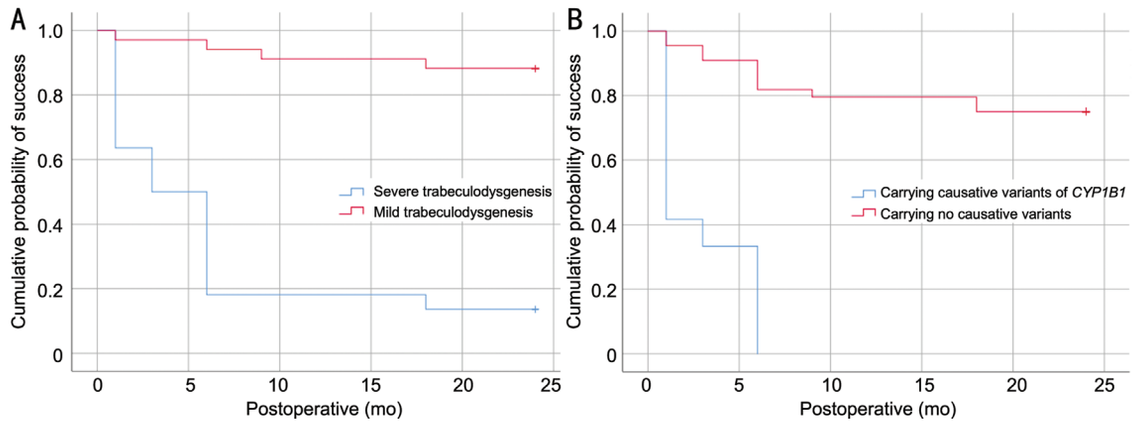

Figure 3 Comparison of surgical success rate of MAT by follow-up time between eyes with two types of trabeculodysgenesis and between eyes with and without causative variants of *CYP1B1* gene A: Kaplan-Meier survival plots of the cumulative probability of success rate between patients with two types of trabeculodysgenesis; B: Kaplan-Meier survival plots of the cumulative probability of success rate between patients with and without causative variants of *CYP1B1* gene. Surgical success was defined as a postoperative IOP≤21 mm Hg without additional medical or surgical therapy. We defined eyes undergoing a secondary surgical procedure as failure and censored them from analysis after the intervention. The differences between and among groups were analyzed using log-rank tests. MAT: Microcatheter-assisted trabeculotomy; IOP: Intraocular pressure; *CYP1B1*: Cytochrome P450 family 1 subfamily B member.

causative variants were marked 1, otherwise 2). Type refers to the 2 types of trabeculodysgenesis under UBM (1, severe type; 2, mild type).

DISCUSSION

Previous studies suggested that trabeculodysgenesis in PCG eyes might prevent the ciliary body from sliding posteriorly, resulting in anterior positioning of the ciliary processes as the anterior chamber angle develops[26-27]. This mechanism explains why anterior insertion of the iris and ciliary muscle into the TM has been recognized both clinically and histologically as a characteristic feature of PCG[16,26,28]. Using 80-MHz UBM, we previously classified this as severe trabeculodysgenesis and linked it to the clinical course and MAT prognosis in PCG[16]. In the present study, we further investigated the genetic basis of this condition in Chinese PCG patients and explored the mechanisms linking severe trabeculodysgenesis with MAT prognosis.

Although the pathogenesis of PCG remains unclear, several genes critical for TM and SC development have been implicated[2]. *CYP1B1* in PCG is generally inherited in a recessive manner with variable penetrance, likely influenced by enzymatic activity levels determined by two-allele variants[26]. Patients with nearly absent CYP1B1 activity due to null genotypes often present with severe trabeculodysgenesis, including poorly developed collector channels, SC, and abnormal ciliary muscle insertion. In contrast, those with mild trabeculodysgenesis, retaining about 60% CYP1B1 enzymatic activity, tend to have preserved collector channels and SC, a more developed TM, and a posteriorly positioned ciliary muscle[27]. Consistent with these findings, our study identified that eight patients (57%) with anterior insertion of the iris and ciliary muscle (classified as severe trabeculodysgenesis) harbored biallelic *CYP1B1* variants, while none with normal insertion (mild trabeculodysgenesis) carried causative variants. This suggests that assessing trabeculodysgenesis using UBM could increase the genetic diagnosis rate for PCG in Chinese patients from 25% (8 out of 32 patients) to 57% (8 out of 14 patients), thereby aiding in genetic counseling.

While previous studies have shown that *CYP1B1* variants contribute to PCG by disrupting TM development[29-30], research also suggests that vascular endothelial cells in *Cyp1b1*$^{-/-}$ mice may fail to undergo capillary morphogenesis[31-32], potentially affecting SC and distal aqueous drainage system. Previous histological findings align with these studies, reinforcing the link between *CYP1B1* variants, severe trabeculodysgenesis, and SC dysplasia[33-34]. Notably, none of our patients with biallelic CYP1B1 variants achieved 360-degree SC catheterization, and all failed MAT. Cox regression analysis further demonstrated that while severe trabeculodysgenesis predicts poor MAT outcomes, the presence of *CYP1B1* variants refines this prediction. These findings further support that causative *CYP1B1* variants may contribute to both severe trabeculodysgenesis and SC dysplasia. Given that MAT success depends on SC morphology and distal aqueous drainage function, patients with causative *CYP1B1* variants may be better suited for filtration surgeries such as trabeculectomy or tube shunt implantation rather than MAT or other angle surgeries[33].

Though we found no variants in four patients with severe trabeculodysgenesis, only one eye achieved 360-degree SC catheterization, and five eyes from these four patients had failed MAT, suggesting other factors may also influence TM,

SC, or aqueous drainage development. Notably, *TEK* gene variants, linked to PCG *via* dominant inheritance, can cause severe SC underdevelopment and elevated IOP[6-34]. However, one patient with compound heterozygous *TEK* variants exhibited only mild trabeculodysgenesis, and his variant-free sister also had PCG, suggesting *TEK* may not be the causative factor. Nonetheless, the patient's MAT failure compared to his sister's success implies a potential role for *TEK* in aqueous drainage beyond TM and SC[20]. Although further research is needed, these findings highlight that gene variants beyond *CYP1B1* may also contribute to SC dysplasia and poor MAT prognosis, even if these cases are less common.

Because of *CYP1B1*'s AR inheritance, single-allele carriers in this study were not classified as having causative gene variants and may harbor other, yet-undiscovered PCG-related variants. However, the prevalence of heterozygous *CYP1B1* carriers challenges a simple recessive inheritance model[9]. Some *CYP1B1* heterozygous variants have been linked to JOAG and primary open-angle glaucoma (POAG), where impaired TM regulation may contribute to disease onset[21-24]. In this study, poor MAT outcomes in patients with the p.Ala330Val variant and JOAG in a father carrying p.Gly61Glu suggest certain heterozygous *CYP1B1* variants may influence glaucoma onset and MAT prognosis. Additionally, the variable expression of CYP1B1 may be due to modifier genes, epigenetic, environmental factors, or developmental events. One patient with *CYP1B1* and *PITX2* variants had severe trabeculodysgenesis and failed MAT, possibly indicating rare *PITX2* variant might increase the severity of the phenotype[25]. Due to the rarity of these cases, a detailed investigation falls beyond the scope of this study. While our findings still highlight the need for family members with *CYP1B1* variants to remain vigilant about their lifelong risk of developing glaucoma.

This study has several limitations. First, while high-resolution UBM effectively evaluates anterior iris and ciliary body insertion, *ex vivo* histological assessment remains the gold standard. Second, the functional impact of detected variants is unknown, with pathogenicity inferred from co-segregation and ACMG scoring. Third, unidentified genes, gene interactions, and incomplete penetrance may influence the phenotype. Fourth, SC catheterization is an indirect marker of SC dysplasia, though histological analysis is impractical. A surgeon with extensive PCG MAT experience minimizes SC identification bias. Lastly, our sample size was limited due to the rarity of PCG, and the analysis was adjusted to account for cases where both eyes of a patient were included.

In conclusion, Chinese PCG patients with severe trabeculodysgenesis as assessed by UBM are more likely to have causative *CYP1B1* gene variants. The combination of severe trabeculodysgenesis with *CYP1B1* gene variants is a valuable predictor of potential SC dysgenesis and MAT prognosis. Given the genetic inheritance patterns, genetic counseling can help assess the risk of recurrence in severe cases, potentially reducing the incidence of these complex cases.

ACKNOWLEDGEMENTS

Foundation: Supported by National Natural Science Foundation of China (No.82201171).

Conflicts of Interest: Gao Y, None; **Lin DT,** None; **Zhou T,** None; **He LH,** None; **Zhao KX,** None; **Yu XW,** None; **Deng L,** None; **Fan ZG,** None; **Shi Y,** None.

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