

Updates on the molecular genetics of primary congenital glaucoma (Review)

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Abstract. Primary congenital glaucoma (PCG) is one of the primary causes of blindness in children and is characterized by congenital trabecular meshwork and anterior chamber angle dysplasia. While being a rare condition, PCG severely impairs the quality of life of affected patients. However, the pathogenesis of PCG remains to be fully elucidated. It has previously been indicated that genetic factors serve a critical role in the pathogenesis of PCG, although patients with PCG exhibit significant genetic heterogeneity. Mutations in the cytochrome P450 family 1 subfamily B member 1 gene have been implicated in PCG and further genes that have been reported to be involved in PCG are myocilin, forkhead box C1, collagen type I $\alpha 1$ chain and latent transforming growth factor β binding protein 2. The present review aims to provide an up to date understanding of the genes associated with PCG and the use of molecular technologies in the identification of such genes and mutations. This may pave the way for the development of preventative methods, early diagnosis and improved therapeutic strategies in PCG.

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1. Introduction

Glaucoma is a disorder of progressive death of retinal optic neurons, which results in a characteristic appearance of the optic disc and visual loss (1). Glaucoma is estimated to affect ~66 million individuals worldwide, with glaucoma patients in Asia accounting for almost 50% of all cases (2,3). A total of 1.2% of children in Britain suffer from glaucoma, and in India, 3-7% of children suffer from this disease (4,5). Based on the modern classification system, primary glaucoma may be classified into three different subtypes: Primary open-angle glaucoma, primary angle-closure glaucoma and primary congenital glaucoma (PCG). PCG is a rare form accounting for 1-5% of all cases of glaucoma (6). In addition, PCG is common, with a prevalence of >32% in children who suffer from glaucoma (7). The overall incidence of PCG in Denmark is 4.8 in 100,000 live births (8).

PCG is a serious form of glaucoma, which results in congenital developmental abnormalities with optic nerve degeneration and dysplasia of the anterior segment that may even contribute to irreversible blindness. The onset of PCG may be as early as at birth but may potentially manifest within the first 3 years of life (9). Numerous patients have congenital anomalies in the anterior chamber angle architectures when they are born. The incidence of PCG varies in different countries and by ethnicity. PCG occurs in 1:30,000 individuals in Australia (10) and 1:24,941 individuals in China (11). In western

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countries, the incidence ranges between 1:10,000 and 1:70,000 individuals (12). In the highly consanguineous population of Saudi Arabia, a minimum incidence of 1:2,766 has been reported, while it is markedly higher in Southern India and in the Roma population of Slovakia, where its incidence lies between 1:1,250 and 1:3,300 (13). Therefore, the occurrence of this disease appears to be associated with consanguinity. The prevalence of PCG-associated blindness is ~10% in Douala, 4.2% in southern India and 6.3% in China (14). However, the pathogenesis of this disease remains to be fully elucidated and requires further study.

Early diagnosis and prompt treatment are particularly necessary for patients with PCG to maintain visual function throughout their lives. Identifying the pathogenic mechanism of PCG may assist in the development of treatments to slow down the development of consequent blindness. The pathogenic factors of PCG primarily involve two aspects, environmental and heredity. Environmental factors include viral and parasitic infections and the use of drugs by the mother during pregnancy. Patients with PCG frequently have a family history of occurrence and familial aggregation. In recent years, specific genes associated with PCG have been identified through the use of molecular genetics-based techniques, including linkage analysis, exon sequencing and comparative genomic hybridization, e.g. cytochrome P450 family 1 subfamily B member 1 (CYP1B1), myocilin (MYOC), forkhead box C1 (FOXC1) and collagen type I $\alpha 1$ chain (COL1A1). The present review is focused on the genetic aspects of PCG occurrence and screening of PCG-associated genes.

2. Clinical characteristics of PCG

Due to the limited cooperativeness of children, no obvious symptoms may be detected at the early stage and examination equipment is mainly designed for adults; missed or delayed diagnosis or misdiagnosis are therefore common (15). Patients with PCG always have an abnormal structure of the trabecular meshwork (TM) and anterior chamber angle, and they present with an increase of the intraocular pressure and a further extension of sclera, optic nerve and associated structures. 'Bull's eye' is a typical clinical feature of PCG. The other common symptoms associated with PCG include epiphora, photophobia, convulsions in the eyelids and buphthalmos (16).

Due to irritation of the cornea, patients with PCG frequently suffer from corneal epithelial edema and haze. The accumulation of aqueous humor leads to disruption in corneal endothelial integrity (17). A markedly thinned iris with reduced iris folds due to reduced stromal thickness and a severe flattening of the anterior limiting layer with a significant positive correlation with increased intraocular pressure (IOP) were reported in patients with PCG (18,19).

Patients with PCG exhibit corneal edema, increased corneal diameter, damaged Haab's striae or an enlarged axial length (20). In newborns, the normal horizontal diameter is 9.5-10.5 mm, while the corneal diameter in children aged 1 is 10-11.5 mm (21). Any increase in corneal diameter (>12 mm) in the first year of life should be noticed by pediatricians (22). The pathological features of PCG are as follows: i) Overlap of ciliary body and TM with the iris; ii) thickened trabecular beams in the trabeculum; and iii) absence of Schlemm's canal (SC) (23).

3. Treatment strategies for PCG

The common treatments for PCG include surgery and drug therapy. Carbonic anhydrase inhibitor is an important medication in the clinic with an efficiency to reduce the IOP by ~25% and fewer general side effects compared with β -receptor blockers (24). But long-term usage may cause serious adverse reactions, including as anorexia, renal acidosis, thirst, fatigue and growth stagnation (25). Furthermore, certain drugs, e.g. pilocarpine, may result in spasm of the ciliary muscle and myopia in pediatric patients (26). At present, the most promising treatment strategy for PCG is surgical intervention combined with drug therapy. However, certain surgical treatments, including filtration surgery, still have disadvantages that may affect the quality of life of patients with PCG. The filtering bleb scar arising from filtration surgery may have an effect on IOP control (27). Furthermore, in certain cases, the visual function damage continues to progress after surgery.

Gene therapy refers to transferring genetic material into individuals with the aim of curing a disease or improving the clinical status of a patient. The major purpose of gene therapy is to replace non-functional or defective genes with new genes that are fully functional so that the gene expression level may revert to its normal state (28). The advances in gene therapy hold significant promise for the treatment of ophthalmic conditions, particularly in PCG. Several studies in animal models have confirmed that gene therapy has efficient effects on aqueous humor and may exert optic ganglion cell protection. Perkins *et al* (29) indicated that treatment of the fascia fibroblasts in a rabbit model with human p21 gene may inhibit cell proliferation and increase the anti-fibroproliferative ability in glaucoma-filtering surgery. This result was also affirmed by Wen *et al* (30). Heatley *et al* (31) transferred human p21 gene into a high-IOP monkey model with recombined adenovirus vector and indicated that the side effect of anti-metabolic drugs (mitomycin C) in filtration surgery had been inhibited. Those pieces of evidence suggest that gene therapy may be suitable for patients with PCG. However, the accurate gene targets, namely the pathogenic genes of PCG, require to be determined.

4. Recent advances in technologies for PCG-associated gene identification

To identify disease-associated loci, the Human Genome Organization generated a specific nomenclature for glaucoma-associated genetic loci in 2011 (32). 'GLC' is a general nomenclature for gene loci in glaucoma. The numbers '1, 2 and 3' refer to the type of primary glaucoma (open-Angle, closed-Angle and congenital/infantile glaucoma, respectively). 'A, B, C and D' indicate the chronology of the mapped genes. Analyses of genetic markers in pedigrees have mapped a glaucoma-associated gene to a region of chromosome 1q, which was termed GLC1A (33). Linkage studies of glaucoma of other pedigrees have mapped PCG-associated genes to chromosome 2p21-22 (GLC3A), 1p36.2-36.1 (GLC3B) and 14q24.3 (GLC3C). The technologies available for the study of the genetics of PCG include sequence analysis, targeted gene mutant analysis and deletion/duplication analysis. Technologies for the identification of PCG-associated genes include linkage analysis,

exome sequencing, whole-exome sequencing and comparative genomic hybridization, which are discussed further below.

Linkage analysis. Linkage analysis is based on familial research and is primarily used for monogenic diseases (34). Linkage analysis was the primary tool used to map the genetics of PCG. The first PCG gene locus, located at 2p21 (GLC3A), was observed in 11 Turkish families with PCG and identified by genetic linkage analysis in 1995 (35). The second chromosomal site to be identified to be associated with PCG is 1p36 (GLC3B), which was identified in eight families with PCG (36). In 2002, a third locus was identified on 14q24 (GLC3C) (37). The likelihood of odds (LOD) score is a basis for the linkage analysis to calculate the logarithm of the likelihood ratio. A LOD score ≥ 3 is considered to indicate a link. However, patients with PCG are rare with a large pedigree; thus, the utility of LOD scores may be limited.

Exome sequencing. Exome sequencing is a technique for identifying mutations in known disease-associated genes through PCR amplification, sequencing and sequence alignment (38). It is used to capture DNA in the exome region and determine the existence of base variations (39). Sheikh *et al* (40) identified two novel mutations, a missense mutation (c.107G>A) and a 12-bp deletion mutation (c.198-209delGGGCCAGGCGGC) in the CYP1B1 gene in a Pakistani family with PCG using exon sequencing. In 2015, Micheal *et al* (41) identified four homozygous CYP1B1 mutants (p.Ala288Pro, p.Asp242Ala, p.Arg355* and p.Arg290Profs*37) in 39 families with PCG using exome sequencing.

Whole-exome sequencing. Whole-exome sequencing is a high-throughput genome analysis method based on exon sequencing. Whole-exome sequencing is currently used for the identification of pathogenic genes and mutated sites in various diseases, including digestive tract tumors and melanoma, mental retardation, primitive dwarfism and idiopathic pulmonary hypertension (42). Due to its high efficiency, this method is applied in prenatal diagnosis, particularly for researching rare mutations in small families and sporadic cases. In 2012, Lim *et al* (43) screened 17 CYP1B1 variations in 57 patients with PCG in the US using whole-exome sequencing.

Comparative genomic hybridization (CGH). CGH is a genomic analysis technology used for the detection of copy number variations (CNVs), which serves an important role in human evolution, genetic diversity and disease susceptibility (44). In 2011, Akarsu *et al* (45) analyzed 25 CNVs (5 deletions and 20 duplications) in 12 Korean patients with PCG using CGH and indicated that the incidence of rare gene-containing CNV frequencies in patients with PCG was 5-30%. Abu-Amro *et al* (46) identified two 7p heterozygous duplications and a 4p homozygous microdeletion in a female Saudi Arabian patient with CYP1B1-negative PCG. In addition to gene copy numbers, studies have indicated that chromosomal aberrations are another possible cause of PCG. Broughton *et al* (47) reported on the pericentric inversion of chromosome 11 in a pedigree affected by PCG and bilateral corneal disease. Nakane *et al* (48) described that a 6p subtelomere deletion occurred in a patient with PCG, severe mental retardation and growth impairment. Merritt and Lindor (49)

detected a duplication on 7q11.23 with CGH in a family suffering from PCG. As the lowest limit of DNA fragment analysis is 3-5 Mb for CGH, if the DNA amplification level is extremely low or a small DNA fragment is lost, this method is not able to detect it due to a lack of sensitivity.

5. Genetic aspects and mutations in genes associated with PCG

A high level of heterogeneity has been noted in the disease-associated loci in patients with PCG, penetrance defects and prevalence of the disease among different populations. PCG is an autosomal recessive disease with variable penetrance (50,51). In 1970, Rasmussen and Philip (52) reviewed the literature on the heredity aspects of congenital glaucoma and indicated that PCG exhibited bilateral heredity in monozygotic male twins; this was later confirmed by Fried *et al* (53). Genetic analysis of the families of patients with PCG suggested that the incidence in monozygotic male twins was higher compared with that in dizygotic twins (54). Therefore, it has been proposed that the inheritance of PCG includes an autosomal-recessive and sex-associated element with variable penetrance. In general, three PCG genetic loci have been implicated in different geographic locations worldwide. Although the exact pathogenic gene and pathway have remained to be confirmed, several implicated genes, including CYP1B1, appear to participate in anterior segment development and they may be involved in the development of PCG (Table I). The CYP1B1 gene is the most studied gene with over 150 variants in PCG. Furthermore, certain evidence provided that the angiotensin 1 (ANGPT)/TEK receptor tyrosine kinase (TEK) pathway may take part in the pathogenesis of PCG.

CYP1B1 gene. CYP1B1 (previously known as glaucoma 3, primary infantile) encodes a homonymous protein (cytochrome P450, family 1, subfamily B and polypeptide 1), and is composed of 3 exons (3, 711,044 and 3,707 bp in length) and two introns (390 and 3,032 bp in length) (Fig. 1) (55). The protein is a member of the B subfamily of cytochrome P450 1, which catalyzes the NADPH-dependent mono-oxygenation of xenobiotics and endogenous molecules (56). The CYP1B1 gene is expressed in various tissue types in the human body, including in the cornea, ciliary body, iris and retina, and may participate in the development of TM (57). CYP1B1 is also expressed in early embryos in several species during the development of ocular tissues (58). CYP1B1 gene mutations may impair the enzymatic activity and stability and reduce localization of the protein to the mitochondria (59,60). CYP1B1 expression is regulated by aromatic hydrocarbons, adrenocorticotropin and peptide hormones (61).

The spectrum of CYP1B1 mutations varies widely across different populations (62). Pathogenic CYP1B1 alleles have been identified in 20% of PCG cases in Japanese patients and 14.9% of patients from the US. Similarly, the rate of CYP1B1-mutated alleles is ~15.2% in Chinese patients with PCG, considerably lower than the percentage in patients from Morocco (47.7%) (63) and Saudi Arabia (75.9%) with mutated CYP1B1 alleles (46). Biallelic variants of CYP1B1 are well known as the genetic cause of PCG, accounting for 22% in Australian patients with PCG (64).

Table I. Genetic loci and genes associated with the pathogenesis of PCG.

Locus	Chromosomal region	Gene	Functions	Pathway implicated in PCG
GLC3A	2p21-22	CYP1B1	Endogenous steroid metabolism	Retinoic acid-independent pathway ^a
GLC3B	1p36.2-36.1	Unknown	Unknown	Unknown
GLC3C	14q24.3	LTBP2	Extracellular matrix organization and formation	BMP/TGF- β pathway ^a
Undesignated	1q24.3-q25.2	MYOC	Trabecular meshwork inducible glucocorticoid response protein; compound variations with CYP1B1	IL-1/NF- κ B inflammatory stress response ^a
Undesignated	17q21.33	COL1A1	A core component of the extracellular matrix in ocular tissues, such as TM and SC	Unknown
Undesignated	6p25.3	FOXC1	Development of anterior segment	Unknown
Undesignated	8q23.1	ANGPT1	Effects on pro-angiogenic and vascular stabilizing through activation of TEK	ANGPT/TEK signaling pathway
Undesignated	9p21.2	TEK	Formation and homeostasis of SC	

^aImplicated in glaucoma, but still unknown in PCG. PCG, primary congenital glaucoma; CYP1B1, cytochrome P450 family 1 subfamily B member 1; LTBP2, latent transforming growth factor β binding protein 2; MYOC, myocilin; COL1A1, collagen type I α 1 chain; FOXC1, forkhead box C1; TEK, TEK receptor tyrosine kinase; ANGPT1, angiopoietin 1; BMP, bone morphogenetic protein; IL, interleukin; TGF, transforming growth factor; TM, trabecular meshwork; SC, Schlemm's canal.

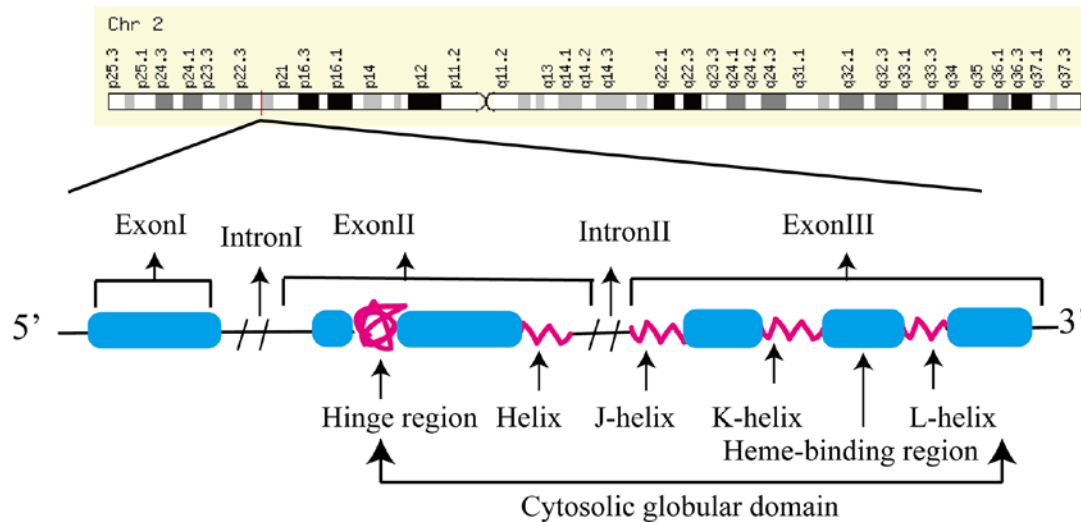


Figure 1. Schematic representation of the CYP1B1 gene. In humans, the CYP1B1 gene, which is composed of three exons and two introns, is mapped on chromosomal location 2p21-22. The coding region of the CYP1B1 gene ranges from the 5' end of the 2nd exon to the last exon. A set of conserved core structures for the heme-binding region corresponds to the carboxy-terminal region of the cytochrome P450 enzyme. Chr, chromosome; CYP1B1, cytochrome P450 family 1 subfamily B member 1.

Libby *et al* (65) demonstrated that developmental defects of TM and SC in eye tissue sections of CYP1B1-knockout mice were similar to those in patients with PCG. Several studies reported that the IOP was increased in CYP1B1-knockout mice (66-68), thus indicating increased oxidative stress levels along with insufficient formation of the periosteum, similar to the alterations observed in human glaucomatous TM tissues. These changes may result in ultrastructural irregular collagen distribution in TM tissue. In Spanish patients with PCG, ~30% of cases carry

loss-of-function CYP1B1 variants, which normally results in a null genotype (69).

Alsaif *et al* (70) identified that c.1405C>T (p.R469W) exhibited a penetrance of 93% in patients with PCG, particularly in Saudi Arabia, followed by a c.182G>A (p.G61E) mutation with 87.7% penetrance. Li *et al* (71) summarized that the primary mutation sites in CYP1B1 gene mutational spectra associated with PCG were G61E, R368H/L, R390H, E387K and R469W, while L385F, R390H and L107V were the most commonly observed sites in China. Ou *et al* (72) indicated that

L107V and R390H were common CYP1B1 mutations with allele frequencies of 3.19 and 3.09%, respectively, in Chinese patients with PCG. Therefore, L107V and R390H may be the most relevant pathogenic mutations in Chinese patients with PCG.

Among the known loci, two variants, which had not been previously associated with PCG, were identified in Brazilian patients (c.182G>A, c.241T>A) (73). Rashid *et al* (74) identified three novel (c.542T>A, c.1436A>G and c.1325delC) and five known (c.868dupC, c.1168C>T, c.1169G>A, c.1209insTCATGCCACC and c.1310C>T) variants of CYP1B1 in 14 Pakistani families. Furthermore, 5 different CYP1B1 variants in 7 families were investigated, indicating that the patients with missense mutations (c.1169G>A and c.1311G>A) had severe phenotypes and poor vision following surgical intervention compared to patients with null variants (75). Talebi *et al* (76) recruited an Iranian family with PCG and discovered three novel CYP1B1 mutations (c.G701>A, c.707delG and c.710delA). These mutations expanded the database of known CYP1B1 gene mutations associated with PCG, which may be useful for genetic counseling and prenatal diagnosis for affected families.

Although >150 CYP1B1 variants have been reported in PCG cases worldwide, they only explain 87% of cases in inbred populations and 25-27% of cases in heterogeneous ethnicities (77).

MYOC gene. MYOC is located on chromosome 1q24.3-q25.2 and contains three exons (604, 126 and 782 bp in length). MYOC encodes myocilin, which is composed of 504 amino acids (78). MYOC is expressed in numerous different types of eye tissue, including TM, sclera, ciliary body and retina. The majority of mutations of this gene identified are located in the third exon and mutations of the MYOC gene may result in structural changes in TM and the ciliary body, impeding the flow of aqueous humor and increasing IOP (78).

Nazir *et al* (79) performed genotyping for rs74315341 and rs879255525 in MYOC in a cohort of 100 patients with glaucoma and 100 control subjects. They demonstrated that the single nucleotide polymorphisms rs74315341 and rs879255525 in MYOC were associated with glaucoma. Millá *et al* (80) identified a novel variation (p.Glu352Lys) of MYOC in 207 Canadian patients with glaucoma; however, its pathogenicity remains to be determined. Recent studies on the MYOC gene are primarily focused on primary open-angle glaucoma. A study in a Chinese pedigree with primary open-angle glaucoma spanning four generations identified a novel mutation (c.1309T>C, p.Y437H) in the MYOC gene (81). Kaur *et al* (82) detected a compound mutation (CYP1B1: c.G1103A, Ar9368His; MYOC: c.G114T, p.Gln48His) with a detection rate of 1.4% (1/72) in PCG. A Gln48His mutation of MYOC was observed in 5 patients with PCG in India (83), and Kim *et al* (84) identified two novel MYOC mutants (c.L228S and p.E240G) in two Korean patients with PCG (detection rate of 2.4%, 2/85) using a bi-directional sequencing method. Chen *et al* (85) hypothesized that MYOC gene mutants may act in association with CYP1B1 through compound variants in patients with PCG, while the mechanism remains to be determined. Although the detection rates of MYOC mutations

are lower than CYP1B1 mutations, they may still explain the pathogenic causes in a proportion of PCG cases.

FOXC1 gene. FOXC1 encodes forkhead box protein C1, which is composed of 553 amino acids. FOXC1 is expressed in the cornea, TM and optic nerve. FOXC1 is present in mesodermal and neural crest-derived cells, including cells of the anterior segment of the eye, and it regulates the external flow and IOP of the aqueous humor. Siggs *et al* (86) noticed that patients with PCG and FOXC1 variants were frequently complicated with systemic features associated with Axenfeld-Rieger syndrome, including hearing loss, heart murmur and developmental delay. Smith *et al* (87) observed abnormal development of the anterior segment (including Schlemm's canal, TM and iris) in mice with a FOXC1 gene mutation and these abnormalities were similar to the phenotypes observed in anterior ocular dysplasia in patients with PCG. Amongst 210 patients with PCG, two heterozygous missense mutations (H128R and C135Y) and three code shift mutations (g.1086delC, g.1155del9 bp and g.1947dup25 bp) were observed in FOXC1 in 5 subjects (2.38%) (88). Medina-Trillo *et al* (89) analyzed FOXC1 variants (rs77888940, c.-429C>G; rs730882054, c.1134_144delCGGCGGCGCGG; rs35717904, c.*734A>T; rs185790394, c.-244C>T; rs79691946, c.*454C>T) in 133 pedigrees with PCG and demonstrated that FOXC1 mutations may affect the formation of goniodysgenesis in PCG. These results suggest that FOXC1 may be associated with the pathogenesis of PCG.

COL1A1 gene. COL1A1 is located on chromosome 17q21.33 and encodes the pro- α 1 chains of type I collagen, whose triple helix comprises two α 1 chains and one α 2 chain. Mutations in the COL1A1 gene are typically associated with skeletal and dermatological conditions, including Ehlers-Danlos syndrome, bone mineral density variation, osteoporosis and Caffey disease (90). Mauri *et al* (91) identified compound heterozygous variants of COL1A1 (p.Met264Leu; p.Ala1083Thr) in 26 patients with PCG by whole-exome sequencing. Collagen protein is a core component of the extracellular matrix (ECM) of the TM, SC and lamina cribrosa, all of which are ocular tissues involved in the development of glaucoma. In addition, pedigree studies have indicated that variants of COL1A1 may affect the development of central corneal thickness and thus result in PCG (92,93).

Latent transforming growth factor (TGF)- β binding protein 2 (LTBP2) gene. LTBP2 is mapped to 14q24.3, located ~1.3 Mb from the GLC3C locus (14q24) (94). The LTBP2 gene consists of 36 exons (95). LTBP2 is expressed in elastic tissues and was determined to be a PCG-associated gene by Narooie-Nejad *et al* (96) and Ali *et al* (97) in 2009. Subsequently, LTBP2 was demonstrated to be localized to the anterior segment and ciliary body. LTBP2 may be associated with PCG due to its putative effect on TGF- β signaling and the ECM of TM. The study of mutations of the LTBP2 gene in patients with PCG thus came into focus. A homozygous variant of LTBP2 (c.895C>T, p.R299X) was identified in two Marfan-like phenotype Roma patients (98). Patients with Marfan syndrome frequently present with lens luxation with a high IOP, which may develop into glaucoma (99). Yang *et al* (100) identified

a nonsense mutation (c.2421G>A, p.W807X) in LTBP2 in 8 Indian patients with PCG. Shazia *et al* (101) identified two mutations in the LTBP2 gene (c.4934G>A, p.Arg1645Glu; c.4031_4032insA, p.Asp1345Glyfs*6) in two Pakistani families with PCG. Kuehn *et al* (102) observed certain symptoms, including increased IOP, globe enlargement of the eye and elongated ciliary body in 8-week-old cats, which had a 4 base-pair insertion in exon 8 of the LTBP2 gene. Inoue *et al* (103) illustrated that the LTBP2 mutants had an abnormal domain structure, which may have resulted in the arrest of secretion of this protein, a process which is essential for the formation of microfibril bundles in ciliary zonules. Consequently, mutations of LTBP2 are a plausible cause of congenital ocular structural abnormalities that may result in PCG; however, the underlying mechanisms by which these mutations lead to PCG remain to be determined.

ANGPT1/TEK: Vascular development in PCG. The ANGPT1 gene is located on 8q23.1 and encodes ANGPT1. ANGPT1 is a secreted glycoprotein, which activates receptors by inducing tyrosine phosphorylation. It serves an essential role in blood vessel maturation and is also involved in the development of SC. Developmental disorders in aqueous humor drainage in the SC and TM were frequently observed in patients with PCG with increased IOP (104,105). The ANGPT/TEK pathway was demonstrated to be involved in the development of SC and aqueous outflow (106,107).

The ANGPT/TEK signaling pathway is composed of 3 ligands (ANGPT1, ANGPT2 and ANGPT4) and its receptor TEK (108). TEK (also known as TIE2) is a receptor tyrosine kinase encoded by the TIE2 gene, which is expressed in endothelial cells. Defects in TEK may result in vascular malformations (109). In human eyes, expression of the TEK receptor is notably high in the SC endothelium. ANGPT1 is a vascular growth factor which affects endothelial activation and dysfunction (110). ANGPT1 is the primary ligand of TEK in the iridocorneal angle and is additionally expressed in certain other vascular-supporting cells (111). The ANGPT1 gene exerts its pro-angiogenic and vascular-stabilizing effects through the activation of TEK. ANGPT2, another ligand of TEK, is a cellular context-dependent agonist/antagonist of ANGPT/TEK signaling. ANGPT2 compensates for the loss of ANGPT1 in ANGPT/TEK signaling (112). The third ligand of TEK is ANGPT4, which is a poorly characterized ligand in mouse models (113). ANGPT4 is important for retinal fluid clearance (114).

Commonly, aqueous humor flow produced by the ciliary body is exhausted through TM and SC, which lie on the iridocorneal angle of the eye. Under normal circumstances, when TEK combines with ANGPT1, it may activate signals, and subsequently, the endothelial cells on the inner wall of SC are under the pressure generated by aqueous humor flow and the gradients. As a result, vacuoles form on endothelial cells, while the pressure-dependent outer pouches of endothelial cells create a channel for aqueous humor flow to cross the SC. The ANGPT/TEK pathway preserves the stability of SC and TM functions (Fig. 2). Its inactivation leads to degeneration of TM and SC, which may further develop into irregular canal formation, increased IOP and glaucoma.

Thomson *et al* (115) demonstrated that genetic disruption in the ANGPT/TEK signaling pathway may result

in increased IOP, buphthalmos and possibly even retinal ganglion degeneration. Subsequently, Thomson *et al* (116) determined that loss-of-function of the ANGPT/TEK signaling pathway resulted in severely hypomorphic canals with elevated IOP in humans and in ANGPT-knockout mice. They identified 3 mutations (p.Q236*, p.R494* and p.K249R) of ANGPT1 among 284 patients with PCG. Souma *et al* (117) described 10 heterozygous mutations of TEK in 189 pedigrees with PCG. These results suggested that the ANGPT/TEK signaling pathway may be implicated in the development of PCG. Therefore, numerous genes associated with the ANGPT/TEK signaling pathway may be involved in the development of PCG.

6. Conclusions

PCG is a complex ocular disorder associated with considerable clinical and genetic heterogeneity. It is a type of serious glaucoma, which primarily affects children of consanguineous couples. Although sporadic cases of PCG may occur, the relevant clinical data and genetic studies provide strong evidence to illustrate that PCG is an autosomal recessive disease. At times, PCG may also be accompanied by other rare genetic diseases, including hypoplasia corpus callosum. Genetic abnormalities may be suspected in pediatric patients with PCG and it may be recommended to exclude the presence of other genetic syndromes such as Axenfeld-Rieger syndrome. The pathogenesis and therapy of PCG are a focal point of research regarding this disease. To date, genetic studies have identified several PCG-associated genes and enlarged the gene map of PCG. However, the associated genes and mechanisms underlying the development of PCG have remained to be fully identified.

Patients with PCG are predominantly from small families, which causes difficulties in studying the genetics of this disease. Developed techniques, including molecular genetics technology and applications of whole-exome sequencing and next-generation sequencing (NGS), have a unique value for the study of cases of PCG, particularly in small families. The elucidation of the pathogenesis of PCG may be assisted by the exploration of novel pathogenic genes, identification of mutations and understanding of the functional relevance of these genes and mutations. The 'third generation of high-throughput NGS technology' or single-molecule real-time sequencing has the advantage of being able to analyze short read lengths. With developments in sequencing technology, it may be possible to perform quantitative analyses of full-length genes on an entire transcriptome level in patients with PCG.

Studies on known PCG-associated genes, including CYP1B1, LTBP2, FOXC1 and MYOC and the ANGPT/TEK pathway, may form a basis for potential gene therapies. It is worthwhile to elucidate the association between CYP1B1, LTBP2, FOXC1 and MYOC and the ANGPT/TEK pathway. The genes reported so far do not fully explain the pathogenesis of PCG, and thus, other genes may also be associated with the development of PCG. Further studies are required to fully understand the complex association between the genotype and phenotype of patients with PCG. The use of transgenic and gene knockout animal models may be necessary to study the function of the PCG-associated genes and mutations

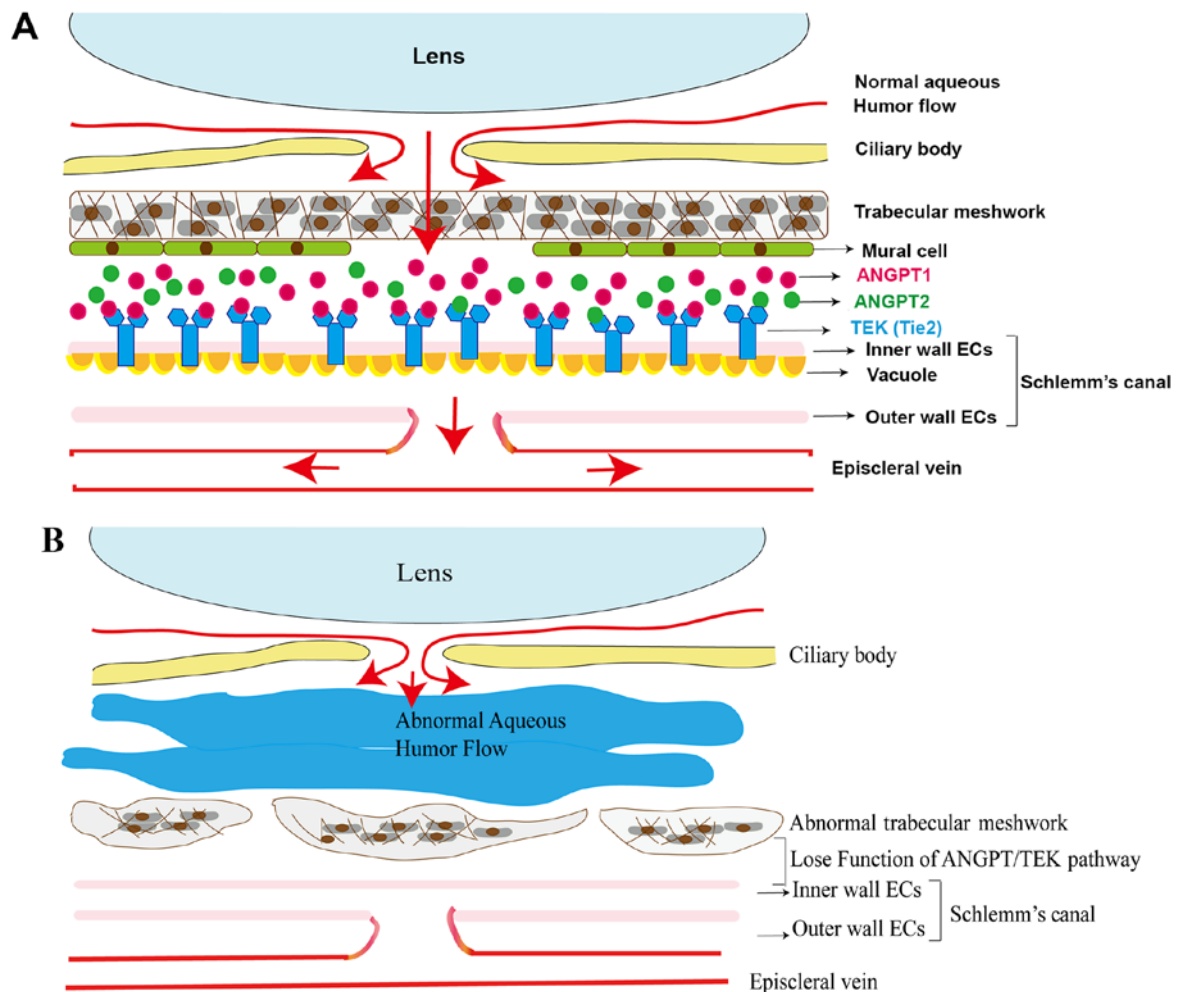


Figure 2. The role of the ANGPT/TEK pathway in SC and TM. (A) Normally, the aqueous humor is drained via the TM and SC-ECs, along with the formation of giant vacuoles. TEK combines with ANGPT1 primarily, while it has limited compensation from ANGPT2. ANGPT/TEK signaling is important for maintaining the integrity and function of SC and thus ensures that the aqueous humor flows out fluently. (B) Absence of continuous ANGPT/TEK signaling results in an increase of EC apoptosis, as well as a decrease of the formation of giant vacuoles and a reduction of the aqueous humor flow going through SC. The accumulated aqueous humor leads to degeneration of the TM and limitation of SC development. TEK, TEK receptor tyrosine kinase; ANGPT1, angiopoietin 1; TM, trabecular meshwork; SC, Schlemm's canal; ECs, endothelial cells.

in vivo and *in vitro*, and elucidate the molecular mechanisms underlying the development of PCG.

Compared with the traditional treatments, gene therapy as a novel therapeutic method has been gradually applied in the field of medicine, providing a broad prospect for the prevention and treatment of PCG. However, it has limitations regarding gene targets, which are used in the treatment of PCG. The potential gene targets used in PCG therapy must to satisfy the following conditions: i) Genes with mutations associated with PCG, ii) gene expression is altered under primary congenital glaucomatous conditions, iii) genes that are known to be involved in pathways recognized as having an effect on IOP or aqueous humor outflow. Gene therapy for PCG is promising but still in the initial stage of experimentation in the field of ophthalmology. Bioinformatics databases and functional genomics may contribute to improved counseling and treatment strategies for patients with PCG.

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Availability of data and materials

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Authors' contributions

CL reviewed literature and wrote the manuscript. DZ designed and revised the manuscript. CL, JZ, HS, QD and XL collected related articles. DZ gave final approval for publication. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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