Review Article

Primary Congenital Glaucoma: Detecting Novel Disease-Causing Variants Using Whole-Exome Sequencing and Pathway Analysis

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Abstract

Primary Congenital Glaucoma (PCG) is an irreversible loss of vision that occurs mainly in infants or in the first years of life. In addition to numerous sporadic cases, PCG exhibits both an autosomal recessive and an autosomal dominant mode of inheritance. The genetic etiology of the disease is not fully understood; however, the role of genes such as CYP1B1, MYOC, LTBP2, and TEK is recognized.

Various molecular biology approaches have been developed to study various diseases' genetic and signaling patterns, especially PCG. Due to the genetic etiology is essential to consider genetic counseling for patients and their families who tend to be at higher risk and eventually reduce the prevalence of ocular diseases.

In this review, in addition to examining various aspects of the disease, we specifically focused on the Molecular and Genetic Basis of PCG, including a small aspect of whole-Exome Sequencing to detect novel disease-causing variants.

Keywords: Cytochrome P-450 CYP1B1; Glaucoma; Genetic Counseling; Whole-Exome Sequencing.

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Introduction

Glaucoma is a class of progressive ocular disorders and the second most common cause of irreversible vision loss. Among them, Primary Congenital Glaucoma (PCG) is an atypical eye disorder due to genetic abnormalities of the trabecular meshwork and anterior chamber angle without other ocular or systemic developmental abnormalities (Figure 1) [1].

Epidemiology

By the epidemiology of PCG as an exceptional disease with the variable incidence in different geographical backgrounds and racial groups, its incidence in the Western world is 1 per 10-20,000 births [2]. The highest incidence found worldwide is 1 in 1,250 in Slovak Gypsies. However, different cultures with higher consanguinity tend to have a higher incidence. However, in certain ethnic groups and countries where consanguinity is common, PCG occurs up to 10 times more frequently [3]. In light of this, we consider one of the most important genes mutated in PCG as there is more information about it in the Middle East. As mentioned earlier, the ratio of CYP1B1 mutations varies widely in different regions, with the Middle East dominating with a percentage of 64.8 % (1:2,500 in Saudi Arabia), followed by the Maghreb (54.4 %) [4, 5], and finally, Iran (comparable figures are not available), where CYP1B1 is the primary mutation and patients are mainly distributed in the north and northwest of Iran (more details about the CYP1B1 gene were presented in the signaling pathways section) [5].

Pathophysiology of PCG

The aqueous humor is the ocular filler fluid in the anterior chamber. It is secreted by the ciliary body and serves to nourish the eye. The primary drainage path for aqueous humor flow is through the posterior chamber. Through the area between the posterior iris and the anterior lens through the pupil to enter the eye's anterior chamber. From there, the aqueous humor exits the eye over the collector canals named trabecular meshwork and Schlemm's canal. Its production ratio to drainage would equalize the intraocular pressure and distend the eye globe. The specific hydrostatic pressure of 15 mmHg

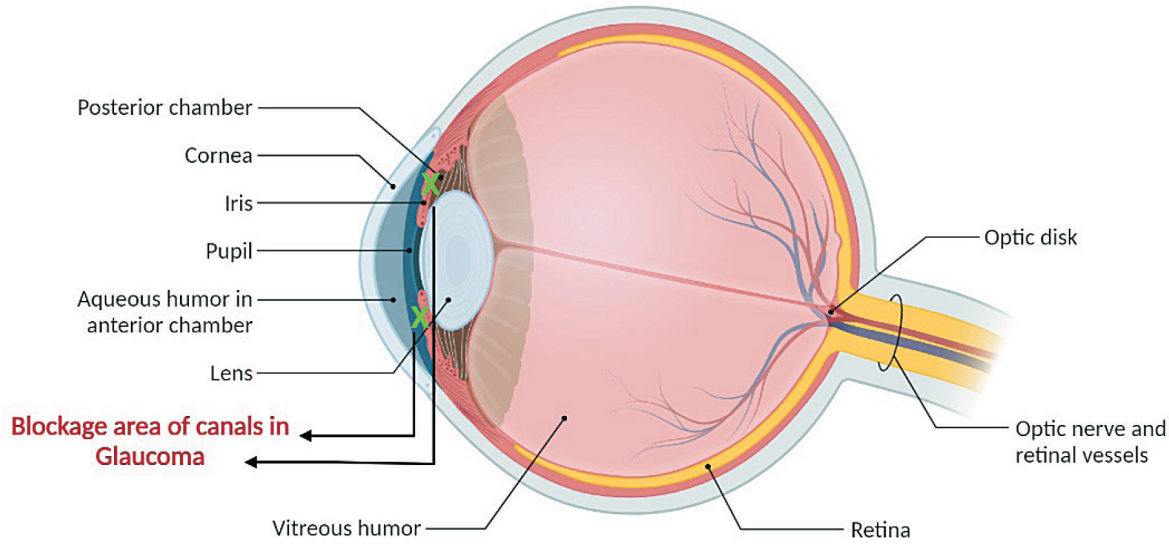

Figure 1: The anatomical and physiological structure of the eye and the area of blockage in Glaucoma "Created with BioRender.com."

keeps the eyeball almost spherical. Therefore, a sharp increase in the production or decrease in the outflow of the aqueous humor leads to dangerous damage to Retinal Ganglion Cells in the inner layer of the globe [6].

Clinical Characteristics of PCG

Typical structural impairments include elevated intraocular pressure (IOP) for the Retinal Ganglion Cells that form the optic nerve. Optic nerve damage can lead to progressive and irreversible defects in the visual field, and elevated IOP levels are often observed in patients (Figure 1) [1]. The clinical symptoms and signs depend primarily on the age and intensity of the disease [7]. The manifestation of PCG typically occurs before the age of three. Therefore, vision loss or pain is not the main complaint. Typical symptoms include photophobia, blepharospasm resulting from the rapid expansion of the child's eye causing buphthalmos, epiphora, horizontal or oblique tears in Descemet's membrane, corneal enlargement, subsequent corneal edema, and opacification [8].

Molecular investigations of PCG

1. Genome studies

Knowledge of the genetic basis of PCG can help clinicians in diagnosis, counseling, management of disease stage, and treatment as it is the most common non-syndromic glaucoma disease in neonates and infants [9]. Among the various genomic tests, Whole-Exome Sequencing has emerged as a method to detect disease-causing variants, specifically in the coding region of the human genome. Many de-novo mutations have been discovered using these methods, particularly in the field of ophthalmology [4, 10, 11]. We aimed to use the WES method to collect novel pathogenic mutations that have been reported in various research papers.

However, apart from being a diagnostic method to identify new pathogenic variants for such a rare disease, WES cannot accurately distinguish all types of genetic variations, especially copy number variants, which may explain some cases that have remained unresolved in studies.

Overall, the combination of epidemiological and WES data analysis results would provide a comprehensive perspective of PCG. This would naturally pave the way to reduce the transmission of pathogenic variants by reducing the number of consanguineous relationships. In other words, pre-marriage genetic testing and consideration of the specific role of migration in reducing the number of patients with ocular disorders are essential.

Autosomal Recessive Inheritance

According to the earliest investigations approach, including genetic linkage analysis of the PCG patients, five loci have been proposed: GLC3A, GLC3B, GLC3C, GLC3D, GLC3E [12].

In order, GLC3A (located at 2p22-p21 chromosome), GLC3B, and GLC3C with locations of 1p36.2-p36.1 and 14q24.3, respectively. GLC3D (14q24.2-q24.3, not overlapping with GLC3C), and lastly, GLC3E (9p21) that the inheritance of PCG at these loci introduced by an autosomal-recessive and sex-associated factor with varying penetrance (Table 1) [13]. Here in each locus that had been specified through linkage analysis before, we aimed to either combine validated single nucleotide variations while obtained through WES procedures in PCG patients and are also related (except other loci sections) to these five loci.

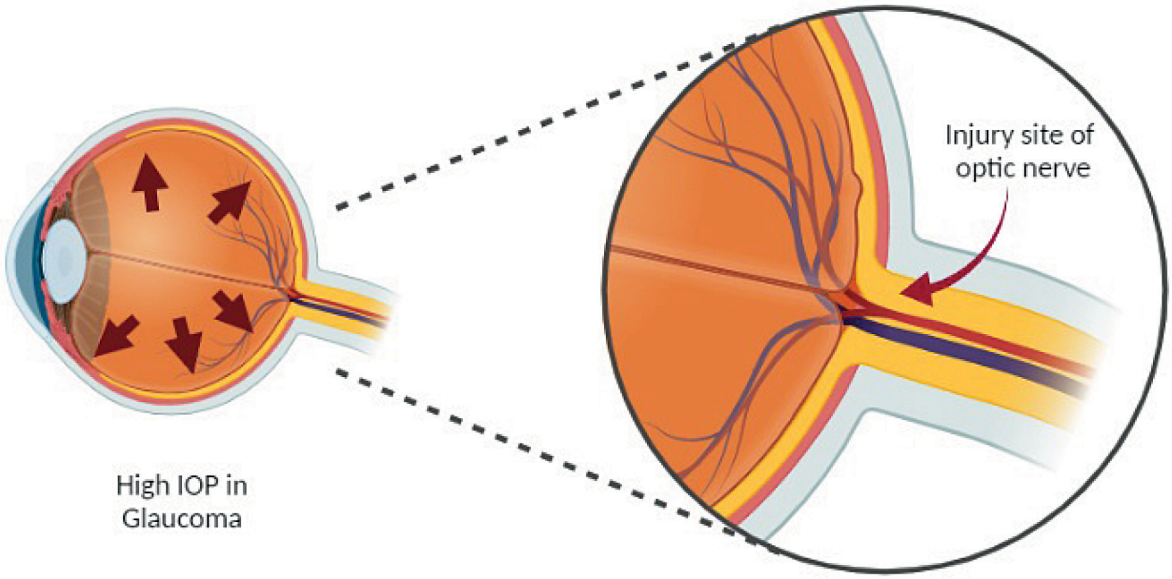

Figure 2: Results of IOP on an eye structure optic nerve and neural cell degradation.

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GLC3A Loci: Contains the cytochrome P4501B1 (CYP1B1gene) at the location of 2p22–21, which was the first reported PCG-causing gene, disease associated with this gene is Primary Congenital Glaucoma 3A (figure3) [7]. The 150 variants illustrated in PCG patients through the CYP1B1 gene are the most studied gene over research related to the PCG. Several studies implicated those mutations in the gene may participate in anterior segment development and may enhance the enzymatic activity and reduce the positioning of the protein to the mitochondria, and, finally, role in the development of PCG [13]. For instance, variations such as rs28936701 (c.1405C > T), rs587778873 (c.1200_1209dup), rs72549380

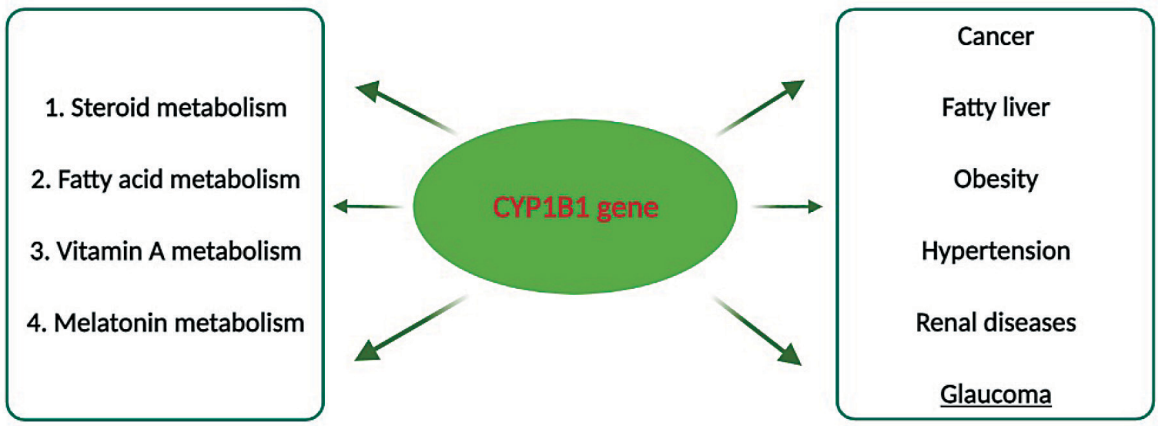

Figure 3: Role of CYP1B1 gene in pathways and related diseases

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Table 1: Genetic loci and genes associated with PCG

Gene	Chromosomal region	Locus	Pathway
CYP1B1	2p21-22	GLC3A	Metabolizing 17-B estradiol pathway
LTBP2	14q24.3	GLC3C	BMP/TGF-β pathway
MYOC	lq24.3-q25.2	-	IL-1/NF-κB pathway
TEK/ANGTP1	9p21.2/8q23.1	-	ANGTP/TEK signaling pathway

(c.1064_1076del), rs771076928 (c.535del), rs28936700 (c.182G > A), rs72549387 (c.171G > A), rs148542782 (c.1168C > T) that lead to change in protein sequences p. Arg469Trp, p. Thr404fs, p. Arg355fs, p. Ala179fs, p. Gly61Glu, p. Trp57Ter and p. Arg390Cys, respectively. It has been multiply reported as pathogenic at highly conserved positions and would be expected to alter substantially or prematurely terminated protein (rs771076928) through the nonsense-mediated messenger ribonucleic acid decay pathway; therefore, impact on the putative enzymatic function of CYP1B1 [14, 15]. Also, three other variants, namely, rs55989760 (c.1159G > A), rs777678299(c.985G > A), rs944452644 (c.349C > T) was reported as a recent potential mutation for PCG in conservative positions change in amino acid sequences p. Glu387Lys, p. Gly329Ser and p. Arg117Trp in order [16]. Likewise, other hotspot candidates were mentioned in the table (table 2).

A novel splice site variant in CYP1B1 (c. [1044-3C > G]; [1064_1076del) results in Arg355Hisfs*69 protein sequence change observed in families from Switzerland, which was the pathogenic variant observed on the CCG CCG haplotype alone [14]. The first CYP1B1 splice site variant in the non-coding intronic region (c.1044-3C > G) was practically analyzed in a cellular system as well [14]. Although the nominal size of this cohort study was a limiting factor, computational analysis observes a reduction in the correct function of the spliced transcript by 98 % compared to the reference; these results would likely occur due to the nonsense-mediated mRNA decay induced by stop codons in the mentioned intron and assumed that this variant is disease-causing [14].

GLC3D Loci: LTBP2 is mapped to 14q24.3 and is located in the anterior segment and ciliary body [13].

Studies suggest that rs121918355 (c. 895C > T) result in p. Arg299Ter mutation in LTBP2 is the major PCG founder mutation in the population of Gypsy while the parents are not known to be related and also in Pakistan [17, 18]. Other variation in LTBP2 rs199581688 (c.1978C > G), rs139018077 (c.1796C > T), rs76172717 (C.2966C > G) were occurred at conservative positions (except rs76172717) orderly arrangement consequences on protein change (Arg660Gly, Pro599Leu, Pro989Arg), although evidences were not enough to role in or out this mutation in disease [19].

By merging two studies of PCG families from Pakistan, two novel mutations have been found, one of them is a missense in LTBP2 (c.107G > A), and another is a 12-bp of deletion (c.198-209delGGGCCAGGCGGC) identified in the CYP1Bl gene using exon sequencing [13]. Two de-novo potential pathogenic mutations (c.4934G > A; c.4031_4032insA), result in p. Arg1645Glu and p. Asp1345Glyfs*6 amino acid change in the LTBP2 gene were observed

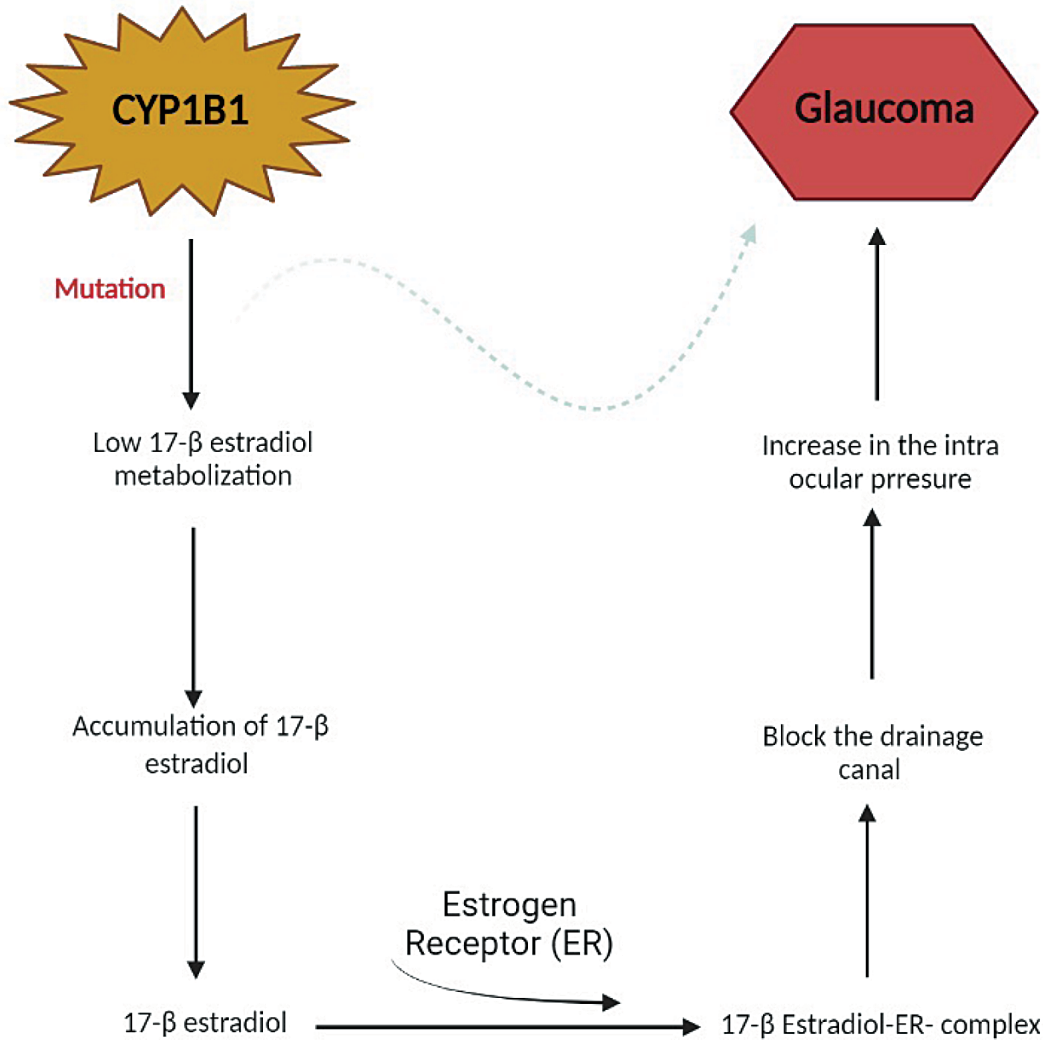

Figure 4: CYP1B1 gene mutation- Steroid metabolism pathway and Glaucoma

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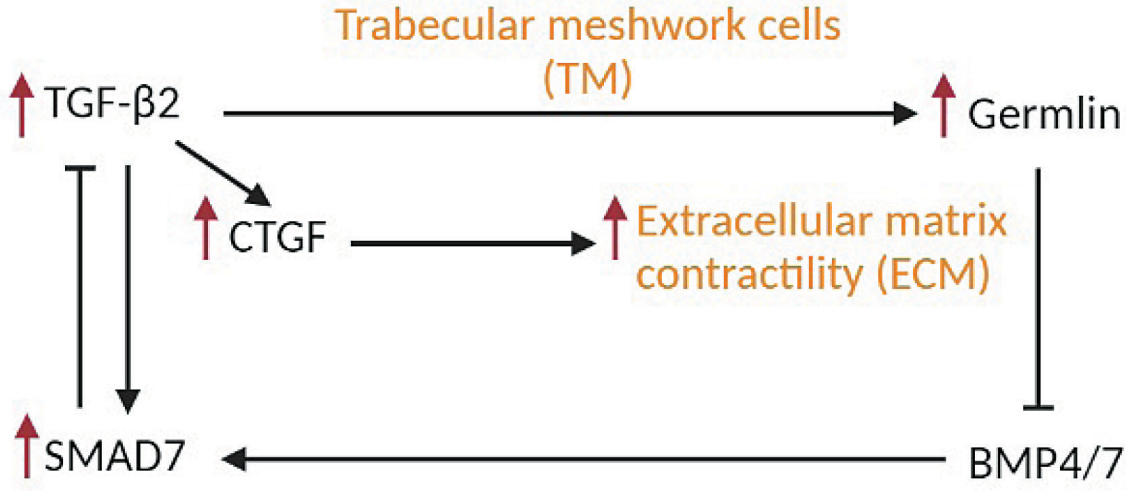

Figure 5: Role of TGF-B/BMP pathway on development of extracellular matrix and trabecular meshwork tissue

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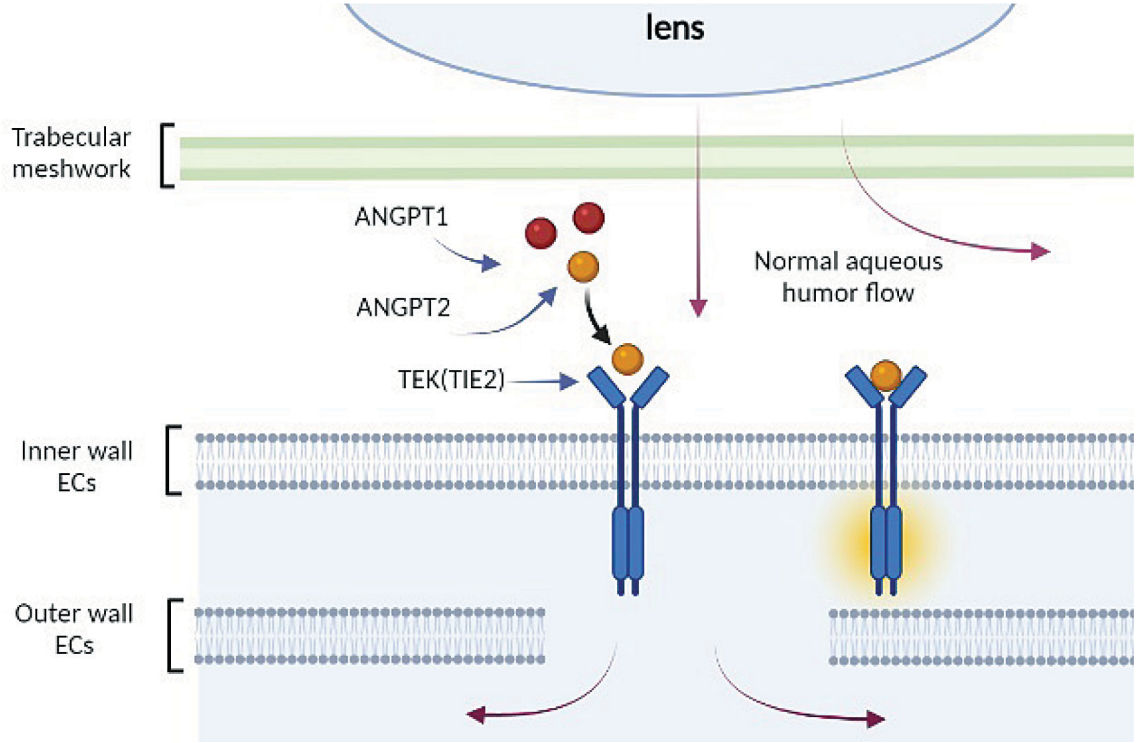

Figure 6: Role of ANGPT1/TEK pathway in ocular tissue
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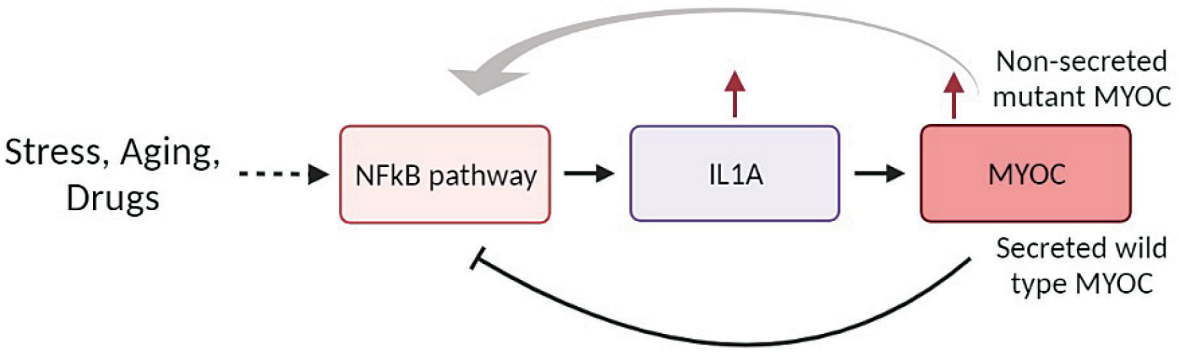

Figure 7: Loop of Wild Type/Mutant MYOC and NFkB/IL1A inflammatory stress
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respectively [20].

GLC3E Loci: Includes the internal membrane of endothelial cell kinase (also named as TIE2) gene, which is expressed in endothelial cells; the other gene ANGPT1 is located on 8q23.1 (table 2) [13].

Other Loci: Although there was no clear information about GLC3B and GLC3C, we introduced genes unrelated to the five specific loci. Alteration in a pair of other potential genes, MYOC and FOXC1, have been observed in small numbers of PCG patients [9]. The location of MYOC is on chromosome lq24.3-q25.2 and encodes Myocilin [13]. Mutations of this gene may lead to changes in trabecular meshwork structure and the ciliary body, blocking the flow of aqueous humor and increasing IOP (Figure 7) [13]. Whereas one or more genes may afflict patients, and the debated genes may regulate or interface with each other.

Table 2: Hotspot variation of PCG according to databases

Gene	Location	Molecular consequence	cDNA location	Protein change	Pathogenicity	Function	Accession
CYP1B1	2p22.2	missense	NM_000104.3:c.1405C > T NC_000002.11:g.38298092G > A NC_000002.12:g.38070949G > A	p.Arg469Trp	Pathogenic	Conservative position- In-silico analysis: damaging on protein structure-function.	VCV000007733
		frameshift	NM_000104.3:c.1200_1209dupTCATGCCACC NC_000002.11:g.38298290_3829829 9dup NC_000002.12:g.38071147_3807115 6dup NG_008386.2:g.9948_9957dup	p.Thr404fs	Pathogenic	Stop signal- Disrupt 140 amino acids Compound heterozygote in several individuals of PCG. Cause loss of protein function.	VCV000068466
		frameshift	NM_000104.3:c.1064_1076del NC_000002.11:g.38298421_3829843 3delNC_000002.12:g.38071278_3807 1290del	p.Arg355fs	pathogenic	Stop signal- Disrupt the last 189 amino acids to 68 incorrect amino acids. Loss of normal protein functions through protein truncation.	VCV000282564
		frameshift	NM_000104.4:c.535del NC_000002.11:g.38301998del NC_000002.12:g.38074855del	p.Ala179fs	pathogenic	Stop signals-Founder mutation in the Moroccan population. Loss of normal protein function due to the protein truncation or nonsense-mediated mRNA decay.	VCV000523943
		SNV	NM_000104.4:c.182G > A NC_000002.11:g.38302350C > T NC_000002.12:g.38075207C > T	p.Gly61Glu	pathogenic	Conservative position between species. Due to the residues differ in polarity, charge, size, and other properties, this variant has impact on secondary structure of protein. In-silico analysis: reduce protein stability-enzymatic activity.	VCV000007730

Gene	Location	Molecular consequence	cDNA location	Protein change	Pathogenicity	Function	Accession
		nonsense	NM_000104.3:c.171G > A NC_000002.11:g.38302361C > T NC_000002.12:g.38075218C > T NG_008386.2:g.5884G > A	p.Trp57Ter	pathogenic	Stop signals- Resulting in truncated protein or absence of protein. Loss of normal protein function due to the protein truncation or nonsense-mediated mRNA decay. Founded as compound heterozygote or bi-allelic in PCG case.	VCV000007737.10
		missense	NM_000104.3:c.1168C > T NC_000002.11:g.38298329G > A NC_000002.12:g.38071186G > A	p.Arg390Cys	pathogenic	Founded as compound heterozygote and homozygote in PCG cases. No publication for the impact of variant. Allele frequency data from public databases did not allow this variant to be ruled in or out of causing disease. But evidence shows pathogenicity in PCG.	VCV000335952
		Deletion	NM_000104.3:c.1536_1541del NC_000002.11:g.38297958_38297963del NC_000002.12:g.38070815_38070820del NG_008386.2:g.10284_10289del	p.Pro513_Lys514del	Pathogenic (single submit)	No publication	VCV000813355.1
		Deletion	NM_000104.3:c.1345del NC_000002.12:g.38071010del NC_000002.11:g.38298153del	P.Asp449fs	Pathogenic (single submit)	No publication	VCV000456639.1
		SNV	NM_000104.3:c.1302G > A NC_000002.11:g.38298195C > T NC_000002.12:g.38071052C > T NG_008386.2:g.10050G > A	P.Trp434Ter	Pathogenic (single submit)	No publication	VCV000456638.1
		SNV	NM_000104.3:c.-285C > T NC_000002.11:g.38303205G > A NC_000002.12:g.38076063G > A	c.-285C > T (5'UTR)	Pathogenic (single submit)	No publication	VCV000813357.1

Gene	Location	Molecular consequence	cDNA location	Protein change	Pathogenicity	Function	Accession
LTBP2	14q24.3	SNV	NM_000428.3:c.895C > T NC_000014.8:g.75022332G > A NC_000014.9:g.74555629G > A NG_021486.1:g.61703C > T	P.Arg299Ter	Pathogenic (single submit)	-	VCV000007554.3
CYP1B1	2p22.2	missense	NM_000104.3:c.1159G > A NC_000002.12:g.38071195C > T NC_000002.11:g.38298338C > T	p.Glu387Lys	Pathogenic/ likely pathogenic	Founder variant in Slovak Romani population. Glu residue is highly conserved. In-silico analysis: Protein function damage- loss of enzyme activity.	VCV000007735
		missense	NM_000104.3:c.985G > A NC_000002.11:g.38301547C > T NC_000002.12:g.38074404C > T NG_008386.2:g.6698G > A	p.Gly329Ser	Likely pathogenic	Completely lack of retinol metabolizing activity.	VCV000632362
		SNV	NM_000104.3:c.349C > T NC_000002.11:g.38302183G > A NC_000002.12:g.38075040G > A NG_008386.2:g.6062C > T	P.Arg117Trp	Likely pathogenic	No publication	VCV000813356.1
TEK	9p21.2	SNV	NM_000459.4:c.578A > G NC_000009.11:g.27169577A > G NC_000009.12:g.27169579A > G	p.Tyr193Cys	Likely pathogenic	No publication	VCV000804257.1
CYP1B1	2p22.2	missense	NM_000104.3:c.1103G > A NC_000002.11:g.38298394C > T NC_000002.12:g.38071251C > T	p.Arg368His	conflicting	Conservative positions between species. In-vitro study: damage/ loss of function in protein-impact on enzymatic activity. Allele frequency is higher than expected for disease in the population. According to not making disease phenotype in all individuals harboring this variant and observed frequently in control population illustrate that it may be pathogenic with low penetrance.	VCV000007739

Gene	Location	Molecular consequence	cDNA location	Protein change	Pathogenicity	Function	Accession
MYOC	1q24.3	missense	NM_000261.2:c.144G > T NC_000001.10:g.171621608C > A NC_000001.11:g.171652468C > A	p.Gln48His	conflicting	The Gln48 residue is conserved. This allele frequency is high but is consistent with the variant being one of the most commonly reported and well-described variants associated with Glaucoma in the South Asian population. Pathogenicity for MYOC related disorders.	VCV000007958
CYP1B1	2p22.2	missense	NM_000104.3:c.958G > T NC_000002.11:g.38301574C > A NC_000002.12:g.38074431C > A	p.Val320Leu	Uncertain significance	Higher allele frequency in the East Asian population. Suspicious details for pathogenicity in PCG.	VCV000225333.4
		missense	NM_000104.3:c.1290C > G NC_000002.11:g.38298207G > NC_000002.12:g.38071064G > C NG_008386.2:g.10038C > G	p.Asp430Glu	Uncertain significance	-	VCV000196208.1
		missense	NM_000104.3:c.155C > T NC_000002.11:g.38302377G > A NC_000002.12:g.38075234G > A	p.Pro52Leu	Uncertain significance	The evidence from publications, in combination with allele frequency data from public databases where available, was not sufficient to rule this variant in or out of causing disease.	VCV000196208.1
		missense	NM_000104.3:c.653A > T NC_000002.12:g.38074736T > A NC_000002.11:g.38301879T > A NG_008386.2:g.6366A > T	p.Asp218Val	Uncertain significance	The evidence from publications, in combination with allele frequency data from public databases where available, was not sufficient to rule this variant in or out of causing disease.	VCV000798154
		missense	NM_000104.3:c.319C > G NC_000002.11:g.38302213G > C NC_000002.12:g.38075070G > C NG_008386.2:g.6032C > G	P.Leu107Val	Uncertain significance	-	VCV000225334
		missense	NM_000104.4:c.367T > C NC_000002.11:g.38302165A > G NC_000002.12:g.38075022A > G NG_008386.2:g.6080T > C	P.Phe123Leu	Uncertain significance	The evidence from publications, in combination with allele frequency data from public databases where available, was not sufficient to rule this variant in or out of causing disease.	VCV000895383.1

Gene	Location	Molecular consequence	cDNA location	Protein change	Pathogenicity	Function	Accession
LTBP2	14q24.3	missense	NM_000428.3:c.1978C > G NC_000014.9:g.74532435G > C NC_000014.8:g.74999138G > C NG_021486.1:g.84897C > G	p.Arg660Gly	Uncertain significance	Evolutionary conservation and computational analysis suggest may have impact on protein.	VCV000828006
		missense	NM_000428.3:c.1796C > T NC_000014.9:g.74535994G > A NC_000014.8:g.75002697G > A NG_021486.1:g.81338C > T	p.Pro599Leu	Uncertain significance	An automated score was calculated to assess if this variant is too frequent to cause the disease. Based on the score, this variant could not be ruled out of causing disease, therefore its association with disease required further investigation.	VCV000314305.4
		missense	NM_000428.3:c.4808G > A NC_000014.8:g.74970002C > T NC_000014.9:g.74503299C > T NG_021486.1:g.114033G > A	p.Arg1603His	Uncertain significance	According to the publications and allele frequency data from public databases, details are not sufficient to rule this variant in or out of causing disease.	VCV000885477.1
		missense	NM_000428.3:c.2966C > G NC_000014.8:g.74978010G > C NC_000014.9:g.74511307G > C NG_021486.1:g.106025C > G	P.Pro989Arg	Uncertain significance	According to the publications and allele frequency data from public databases, details are not sufficient to rule this variant in or out of causing disease.	VCV000775349.4

While there is no well-known pathway to declare the role of FOXC1 in PCG it has been observed abnormal development of the anterior segment in mice which has a FOXC1 gene mutation, and these traits were the same as the phenotypes of patients with PCG; hence, these results illustrate that FOXC1 might be linked up with the pathogenesis of PCG [13]. At last, the location of the COLA1A gene is on chromosome 17q21.33, which encodes the pro-α1 chains of type I collagen [13]. Collagen protein is the main ingredient of the extracellular matrix (ECM) of the Trabecular Meshwork and Schlemm's Canal. Studies have illustrated that variant of COL1A1 may impact the thickness of the central corneal and result in PCG [13].

Autosomal Dominant Inheritance

Recently, it has been revealed that PCG can be transmitted by different levels of inheritance [9], and the disease is inherited in an autosomal dominant manner due to a pathogenic variant in TEK [7].

Di-genic inheritance

According to the clinical reports, there is a di-gene mode of inheritance for CYP1B1 and MYOC, between CYP1B1 and TEK for PCG. In this context, results of studies on hotspot mutations of these genes such as the presence of a Gln48His variation in the homozygous state in a PCG case without a mutation in CYP1B1 or also with compound heterozygous mutations in rs79204362 (c.1103G > A) and MYOC rs74315339 (c.144G > T) in another patient explain that the disease could be caused by a di-genic mode of inheritance in these cases [21].

In summary, all variations across the CYP1B1 gene mentioned in this section are potentially pathogenic, e.g., rs28936701 as a variation in the conservative position or rs587778873 and rs72549380 leading to premature coding of the protein, and finally variations with the conflicting interpretation of pathogenicity (rs79204362, rs74315339), which are referred to as rolling in the disease. For more information on the panels recently obtained for PCG, Primary congenital glaucoma book from the NCBI database - Multigene Panel section [7]. (See Table 2 for more information on all variations).

2. Transcriptome studies

Innovation in systems biology has brought us a variety of computational tools and analytical means [22-25]. This approach successfully helps researchers to investigate molecular mechanisms of inhibition and activation of the genes and transcription factors, predict drug-target interactions, and detect novel molecular biomarkers of phenotypes [26-28].

In one study, the function of the GLIS1 gene was investigated. The gene was found to play a critical regulatory role and be expressed in the trabecular meshwork, a crucial tissue in regulating efflux duration. Alterations in the expression of GLIS1 lead to deterioration of the trabecular meshwork, causing inadequate aqueous humor outflow and increasing IOP. The combination of RNA-Seq and ChIP-Seq identified several genes directly regulated by GLIS1, including MYOC, CYP1B1, LOXL4, and LTBP2, which were previously implicated in glaucoma [29].

Another result showed that the transcripts of genes encoding TSP -1 and PTGS2 were most up-and down-regulated, an increase in TSP -1 (8.66-fold) and a decrease in PTGS2 (9.09-fold) were observed in cases with acute primary angle closure (APAC). The data of expression of TSP -1 and PTGS-2 in separate samples by real-time assay RT-PCR are consistent with

the microarray data. The amount of TSP -1 transcript in Peripheral Blood Mononuclear Cells (PBMCs) was higher (approximately 3.13-fold) in patients than in controls (P value = 0.030). The amount of PTGS-2 transcript was lower, about 8.27-fold (P value < 0.001). TSP -1 is known as a potent activator of TGF-β1, and TGF-β1 has been shown to trigger the expression of TSP -1 in Peripheral Blood Mononuclear Cells (PBMCs) at the mRNA level [30].

3. Proteome studies

TSP -1 is an activator of TGF-β, which is present in healthy tissues in a hidden form. Like TSP -1, TGF-β1 and TGF-β2 are elevated in aqueous humor, trabecular meshwork, or plasma of people with primary Open-Angle Glaucoma (POAG) or exfoliative Glaucoma. To investigate whether gene changes in gene transcripts result in different amounts of protein translation, plasma was experimented with to obtain the levels of TSP -1 and PGE2. A molecule that translates PTGS-2 is also examined for the level of active TGF-β1 in plasma since TSP -1 activates TGF-β1. TSP -1 is a matricellular protein that can interact with various receptors, matrix molecules, or growth factors. Therefore, it plays various roles in cell adhesion, migration, and inhibition of angiogenesis. Expression of TSP -1 is deficient in normal adult tissues but is strongly upregulated in response to injury or tissue remodeling. Consistent with these findings, an increase in active TGF-β1 levels has been found in the plasma of patients with Acute Primary Angle Closure (APAC), as TGF-β signaling leads to fibrosis and a pathological increase in extracellular matrix deposition in various disorders such as Glaucoma [30].

By the function of high IOP in all forms of Glaucoma, these findings could be related to other forms such as the primary congenital form and primary angle-closure Glaucoma and primary open-angle Glaucoma.

Signaling pathways

According to signaling pathways of Glaucoma, some genes have been studied well. The protein coded by CYP1B1 is a part of the B subfamily of cytochrome P450 1, which roles in a hydroxylase for 17b-estradiol, and the productions of these enzymatic reactions contribute to metabolic processes that accumulate 17b-estradiol-ER complex involved in generating Glaucoma (Figure 4) [31]. Also, GLIS1 gene binding peaks in the proximal promoters of CYP1B1 and MYOC are in a region close to the functional AP-1 responsive element. Furthermore, the proximal promoter of CYP1B1 includes a G/C-rich SP1 binding sequence because its resemblance to the consensus sequence of GLISBS might act as a GLIS1 binding site. This suggests that these promoters may have a regulatory function for various transcription factors [29].

Another gene that has been studied is LTBP2. LTBP2 may be associated with PCG because of its assumed effect on TGF-β signaling and extracellular matrix (ECM) of the trabecular meshwork [13] (Figure 5). The putative effect of LTBP2 mutations on the TGF-β signaling pathway has a plausible role in ocular anomalies that may lead to PCG [13].

The ANGPT/TEK signaling pathway consists of 3 ligands (ANGPT1, ANGPT2, and ANGPT4) and its receptor tyrosine kinase TEK in which ANGPT 2/ANGPT4 are compensating for the loss of ANGPT1 that is the prime ligand of TEK in ANGPT/TEK signaling [13]. The ANGPT/TEK signaling pathway is important for maintaining the integrity and function of Schlemm's canal and thus ensures that the aqueous humor flows out fluently. Lack of

ANGTP/TEK signaling continually increases apoptosis of endothelial cells and reduces the aqueous humor flow through Schlemm's canal [13]. Lastly, due to degeneration of the Trabecular Meshwork, accumulation of the aqueous humor and limitation of Schlemm's canal development occurs, inevitably leading to PCG (Figure 6) [13]. A study hypothesized that the different spectrum of mutations in the MYOC gene would create stress and activate the NF-kappaB pathway [32].

Conclusion

Overall, early and accurate diagnosis of PCG is critical, as appropriate treatment could be considered before optic nerve impairment and vision loss occurs.

Due to the advantages of sequencing methods, especially diagnosis based on molecular findings from WES and its substantial diagnostic role in observing a wide range of variants alongside bioinformatics research on various aspects and functions of these variants in patients with PCG, whether the additional data of genetic variants could further refine the risk of the pervasiveness of this disease across generations or is merely collecting data would be a crucial question to be examined to find an accurate answer. However, data collection advances scientists' knowledge of the full spectrum of the disease. It could also play a role in the development of a specific diagnostic panel by considering potential variations, particularly in the CYP1B1 gene with different pathogenic behaviors, that play a crucial role in PCG and could also be used for early and accurate detection, which should be essential for preventing the development of disorders such as Glaucoma.

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Footnotes and Financial Disclosures

Conflict of interest

The authors have no conflict of interest with the subject matter of the present manuscript.