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EXPLORING GENETIC DETERMINANTS OF PRIMARY CONGENITAL GLAUCOMA IN PAKISTANI COHORTS

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Abstract

Glaucoma is the second leading cause of blindness, affecting approximately 65 million people globally. Primary congenital glaucoma (PCG) is a hereditary disorder that arises during the development of the eye, especially in the anterior chamber angle and trabecular meshwork. It causes irreversible childhood blindness due to increased intraocular pressure, corneal opacity, and optic nerve damage. The three most commonly mutated genes associated with PCG are CYP1B1, LTBP2, and TEK. In Pakistani families, the most screened gene for genetic analysis of autosomal recessive PCG is CYP1B1, accounting for 50% of mutations. LTBP2 mutations have been identified in CYP1B1-negative cases, whereas TEK mutations remain unreported in the Pakistani population. This review summarizes the most common pathogenic variants in these genes to create a database. Moreover, the most susceptible domains of these proteins are determined by Alphafold. Furthermore, it highlights the importance of disease-causing variants studies in any specified location. And it signifies the impact of family history and consanguineous marriages on the higher prevalence of PCG. Understanding the spectrum of PCG in different ethnicities can facilitate the development of diagnostic kits for the early detection of PCG in Pakistan. Early diagnosis and Genetic counseling of families are pivotal in PCG management. Applications of Next-Generation Sequencing and Whole Exome/Genome Sequencing can reveal novel PCG-associated genes/mutations in already known genes even in unsolved cases, opening doors for personalized genetic counseling and precision therapies.

Keywords: *CYP1B1, Disease spectrum, Glaucoma, Inherited eye disorder, Pakistani population, Primary congenital glaucoma*

INTRODUCTION

GLAUCOMA

Glaucoma is a heterogeneous inherited ocular disorder that affects approximately 15% of the blind population in the world (1). Glaucoma comprises a group of neurodegenerative diseases accompanying apoptotic death of retinal ganglion cells (RGS). This slow and gradual degradation of retinal ganglion cells and their exon leads to atrophy of the optic nerve and depreciation of the visual field unless the affected individuals become legally blind (2, 3). This apoptotic death is linked to an imbalance between the secretions and affluence of aqueous humor from the posterior chamber into the anterior chamber of the eye through the assistance of the ciliary body (4).

As we know, the aqueous humor maintains the pressure in the eye. Any irregularity in the fluid raises the intraocular pressure (IOP), which produces mechanical stress on the posterior chambers, especially lamina cribrosa and adjacent tissues (5). Axon damage and interruption in a neuronal signal are the main consequences of this stress (6, 7). In early times, Glaucoma was named a cataract (8). With the passage of time and continuous work, scientists named the enlargement of the eye globe buphthalmias. An increase in IOP in the anterior chamber is the main feature of glaucoma (9).

The precise measurement of IOP is a crucial factor for diagnosis and management of glaucoma. Diagnostic methods include tonometry as a gold standard for IOP measurements, gonioscopy for

monitoring the drainage angle of the eye, and Fundoscopy for the examination of damage to the optic nerve, which is an indicator to assess the severity and progression of glaucoma (10).

CLASSIFICATION OF GLAUCOMA

Nowadays, glaucoma has been divided into three categories.

PRIMARY OPEN-ANGLE GLAUCOMA

In this type of glaucoma, the contact between the iris and trabecular meshwork is normal but there is resistance to drainage of fluid and raises the intraocular pressure in the eye. It usually appears after 40 years in an individual (11).

PRIMARY ANGLE-CLOSURE GLAUCOMA

This is due to blockage between the iris and trabecular meshwork which causes the anterior segment of the eye to be shallower with increased intraocular pressure and movement of lenses at any angle. It is more common in females (12).

PRIMARY CONGENITAL GLAUCOMA

Primary Congenital Glaucoma (PCG) is the inherited abnormality during the development of an eye, especially in the anterior chamber angle and trabecular meshwork, that appears within the first three years (13-16). This is interlinked with disproportion in the intraocular pressure (IOP), following the optic nerve damage declaring the child legally blind (17). Imbalance in IOP demonstrated multiple clinical features of PCG like buphthalmias, corneal opacity, edema, Descemet membrane breakdown, thinning of sclera, deformation of the anterior chamber and iris, optic disc impairment, photophobia, watering eyes, blepharospasm, and epiphora (Fig. 1). Poor or no treatment leads to vision loss in children (18, 19).

The rate of PCG is higher in the Middle Eastern population (1/2500 infants) than in Western countries (1/10000-20000 live babies) because cousin marriages are common (20, 21).

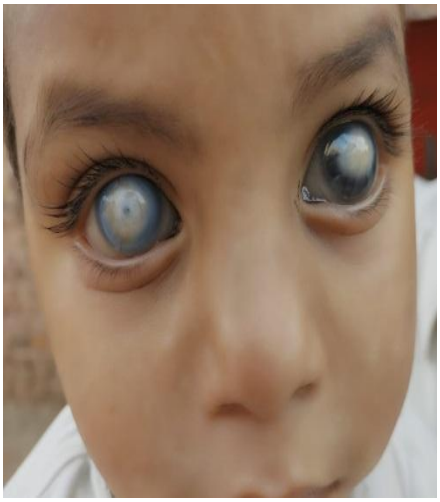


Fig. 1. The primary congenital glaucoma patient has corneal opacity in both eyes and the presence of a brownish ring in the right eye

This review article will discuss the prevalence of primary congenital glaucoma, and the genes associated with PCG in affected individuals. We will also study the most common mutations among patients with Primary congenital glaucoma in the Pakistani context.

GENES INVOLVED IN PCG

PCG is inherited as an autosomal recessive or dominant form. Human cytochrome P450 1B1 (CYP1B1) and Latent transforming growth factor beta binding protein 2 (LTBP2) can cause autosomal recessive PCG (22). Studies show that any mutation in CYP1B1 causes loss of function of the gene and results in improper development of the trabecular meshwork (TM) and disruption in signaling pathways, leading to an increase in IOP, which is an indication of PCG (23-25). More than 150 mutations for CYP1B1

are identified globally. It is the most common cause of PCG in different ethnicities (26, 27). CYP1B1 mutations are higher in South Asia, Africa, the Middle East, and Brazil than in China, Japan, and Indonesia, where the frequency of consanguineous marriages is higher (28). In the Pakistani population, CYP1B1 mutation was first identified for PCG in 1997 (29). Now, it accounts for 50% of autosomal recessive PCG cases (30). R390H is the most common mutation of CYP1B1, identified in the patients of PCG in China, Pakistan, India, and Iran (25, 26, 31, 32). The mutations in LTBP2 are rarely present in the autosomal recessive cases of PCG in Pakistan and Iran (33, 34). LTBP2 mutations are screened in the CYP1B1 negative cases. No mutation of LTBP2 is reported from Northern India, China, the UK, and the US (27, 35-37). Tunica interna endothelial receptor tyrosine kinase (TEK) was reported in autosomal dominant PCG (38). Mutations of TEK have been identified among patients of PCG with ethnicities of American, European, African, Australian, Romani, Latino, Chinese, and Mexican population (38-42). However, no mutation has been reported in the Pakistani population. Next-generation sequencing, whole genome/exome sequencing, will uncover TEK mutations in autosomal dominant cases of PCG in developing countries like Pakistan.

CYP1B1

Human cytochrome P450 1B1(CYP1B1) is expressed in multiple regions of an eye like the cornea, ciliary body, (31), and retina (31, 43-45). The structure of membrane-bound CYP1B1 is comprised of an N-terminal containing a membrane-spanning domain, a proline-rich hinge region, and a highly conserved C-terminal containing J-helix, a K-helix, and a heme-binding region (23, 46, 47).

Mutations in any part of CYP1B1, whether it is the N-terminus or C-terminus, are associated with changes in the confirmation of CYP1B1 and its ability to bind haem. This leads to the malfunction of the CYP1B1 enzyme in the signaling pathways of the eyes in PCG patients (23, 24).

CYP1B1 is an enzyme located in the endoplasmic reticulum. Retinol and 17β-estradiol are its natural substrates. Retinol substrate activity of CYP1B1 is linked with PCG (48). To date, the exact mechanism of CYP1B1 in PCG patients is a mystery to scientists. It has long been assumed that it plays a role in metabolic processes for ocular differentiation, such as the development of the anterior segment and the trabecular meshwork.

It has been suggested that CYP1B1 mutation-induced gene product loss-of-function contributes to the TM of PCG. Toxic metabolites are removed by the CYP1B1 enzyme, which also metabolizes a signaling molecule necessary for the development of ocular tissues (possibly endogenous steroid metabolism as shown in Fig. 2 (24, 49-51). In the meanwhile, MYOC and 17 estradiol, a CYP1B1 metabolite, influence TM formation by participating in the cAMP/protein kinase A pathway (52, 53). However, it is still unclear what the endogenous target in vivo is.

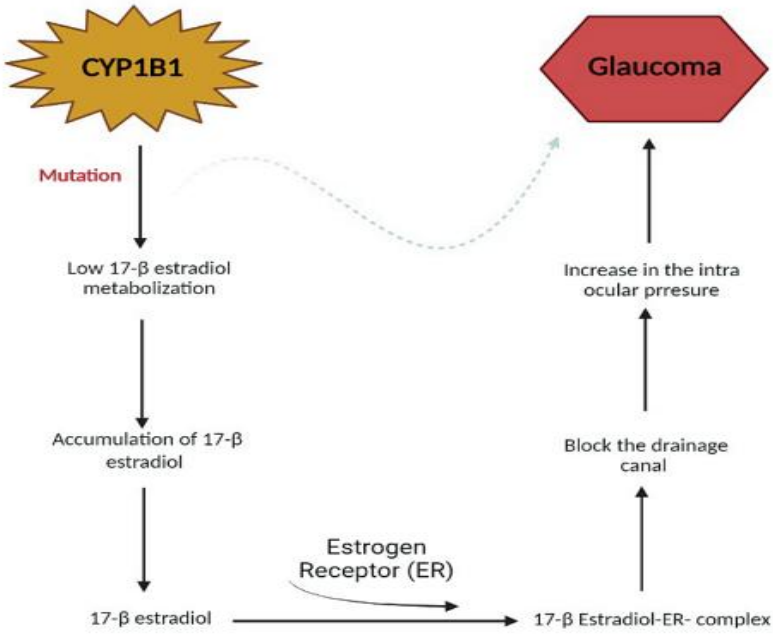
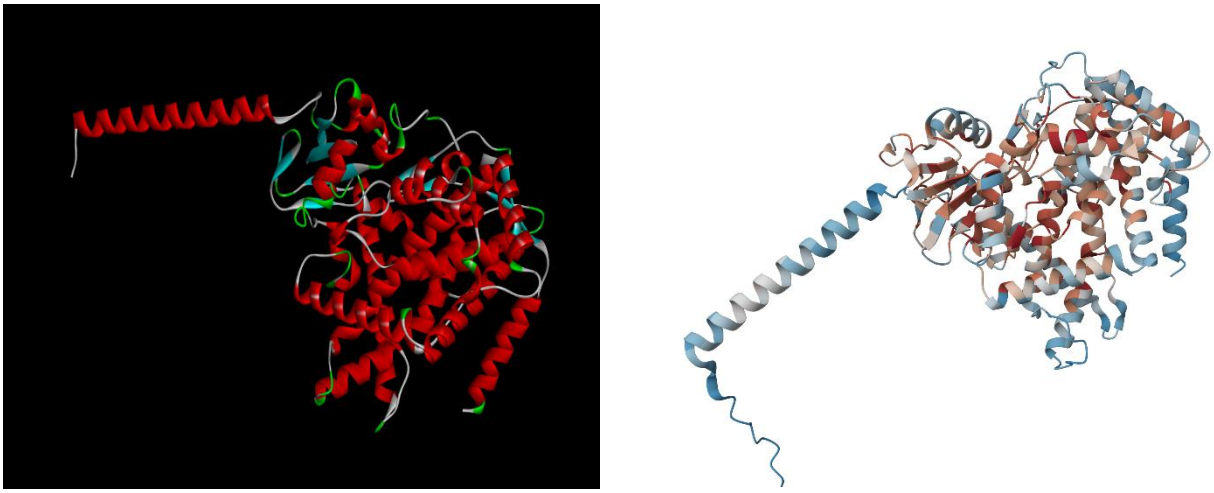


Fig. 2. Pathway showing the mutation in CYP1B1 leads to glaucoma. *Adopted from Zavarzadeh et al., 2020 (51).*

An expression analysis study on increased IOP in CYP1B1 deficient mice revealed that CYP1B1 deficiency causes structural abnormalities in the trabecular meshwork (TM), increased oxidative stress, and reduced levels of postn in young mice. Likewise, Human glaucomatous TM tissues showed elevated oxidative stress and decreased postn expression. Moreover, impaired TM development resulting from CYP1B1 mutations exhibited lower levels of postn and increased IOP in primary congenital glaucoma patients (25, 31, 54). According to another study, CYP1B1 is a crucial redox homeostasis regulator that is connected to elevated oxidative stress in trabecular meshwork cells (TMCs) and retinal vascular cells in PCG (55). Arachidonic acid, a product of CYP1B1, also controls corneal transparency and the activity of the Na+ - K+ ATPase in corneal microsomes (56-58).

CYP1B1 was expressed in the dorsal and ventral retina of the ocular fissure of zebrafish, and it overlapped with RALDH2 and RALDH3 (59, 60). The dehydrogenase-independent route converts vitamin A into the retina and subsequently into RA with the aid of CYP1B1 (59). Colobomatous abnormalities were caused by overexpression of CYP1B1, which prevented the closure of ocular fissures (60). As opposed to this, CYP1B1 knockdown alters neural crest (NC) later migration via the RA-independent mechanism and causes the inferior optic fissure to prematurely close (60). Additionally, in CYP1B1-knockdown zebrafish, the restoration of retinal development and the development of the iris stroma produced by the crest were both postponed in the larval stage (58, 61). The structure of CYP1B1 by X-ray crystallography and the most prone regions to mutations using Alphafold are shown in Fig. 3.



(a) (b)

Fig. 3. Structure of CYP1B1 and position of mostly reported mutations. (a) PDB structure of CYP1B1 determined by X-ray crystallography (b) Structure determined by AlphaFold for most vulnerable positions for mutations according to literature. The brown color shows the area where most of the time mutation occurs

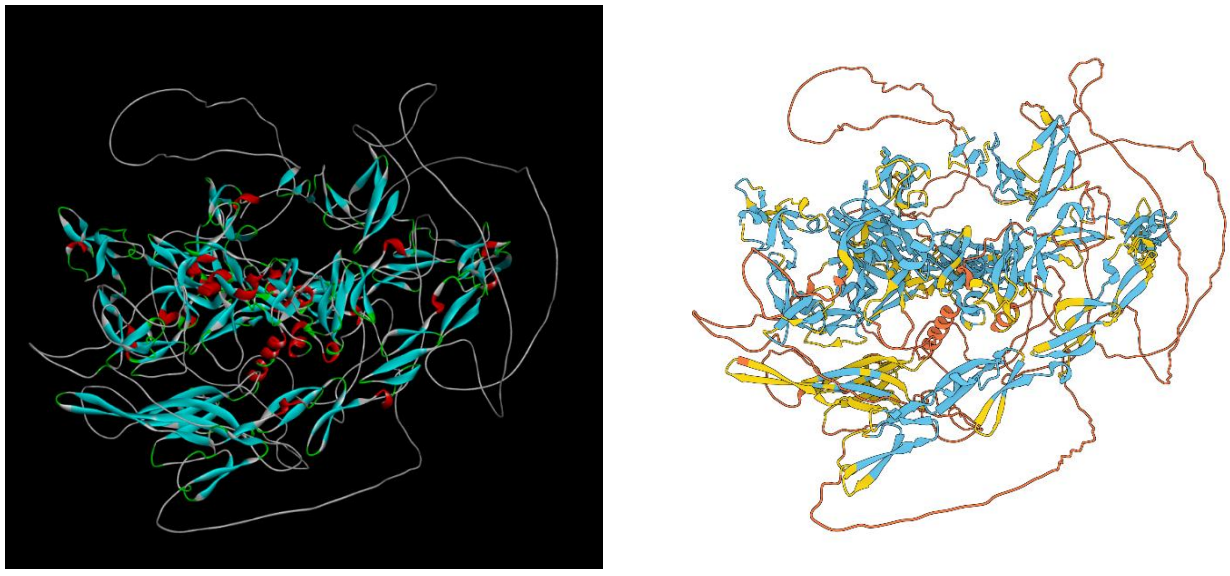
LTBP2

Latent transforming growth factor beta binding protein 2 (LTBP2) is expressed in the trabecular meshwork of the eye and controls the intraocular pressure, growth of ciliary zonules, and production of aqueous humor (62, 63). The structure of LTBP2 is comprised of twenty epidermal growth factor (EGF)- like domains having calcium-binding motifs for protein-protein interactions, four transforming growth beta binding protein (TB)-like modules having eight cysteine residues that give conformation flexibility, and an amino-terminal peptide. The C-terminal of LTBP2 interacts with Fibrillin 1(FBN1) directly to play its role in the extracellular matrix. LTBP2 competes with LTBP1 for this binding (33).

The mutations in the LTBP2 sequence cause conformational changes in its structure and lead to primary congenital glaucoma in patients of Asian origin e.g., Pakistan, Gypsies, Iran, and India (33, 34, 64, 65). The mechanism behind the involvement of PCG is unknown. However, the expression of LTBP2 is higher in TM, the frontal chamber of the eye, and the ciliary zone microfibrils. Multiple disease-causing variants of PCG highlighted its importance in maintaining the extracellular matrix integrity (35, 66). LTBP2 protein plays a crucial role in regulating the TGFβ1 signaling, which is essential for the initiation of downstream transcription factors via phosphorylation and activation of TGFBR1 following TGFβ1-TGFBR2 interaction (67). This protein is important in producing elastin fibers during tissue morphogenesis (33, 63,

65, 68). It is hypothesized that pathogenic variations in LTBP2 lead to structural changes in the protein, inhibiting the binding of FBN1, thereby disrupting microfibril architecture (69). Moreover, LTBP2 null mice studies revealed lens dislocation, likely resulting from the disorganization of ciliary zonules (70). This asymmetry or anomalies in confirmation may result in high IOP and subsequent retinal ganglion cell apoptosis, which are hallmarks of glaucoma (68).

LTBP2 pathogenicity is involved in other eye defects like Marfan syndrome and secondary glaucoma along with its interaction with other genes like the FBN1 gene (33, 65, 71). Few mutations related to LTBP2 for primary congenital glaucoma have been reported in the Pakistani population when there is no mutation figured out in the CYP1B1 gene in the patient (33, 63, 65, 68). The structure of LTBP2 by X-ray crystallography and the most prone regions to mutations using Alphafold are shown in Fig. 4.



(a) (b)

Fig. 4. Structure of LTBP2 and position of mostly reported mutations. (a) PDB structure of LTBP2 determined by X-ray crystallography (b) Structure determined by AlphaFold for most vulnerable positions for mutations according to literature. The brown color shows the area where most of the time mutation occurs

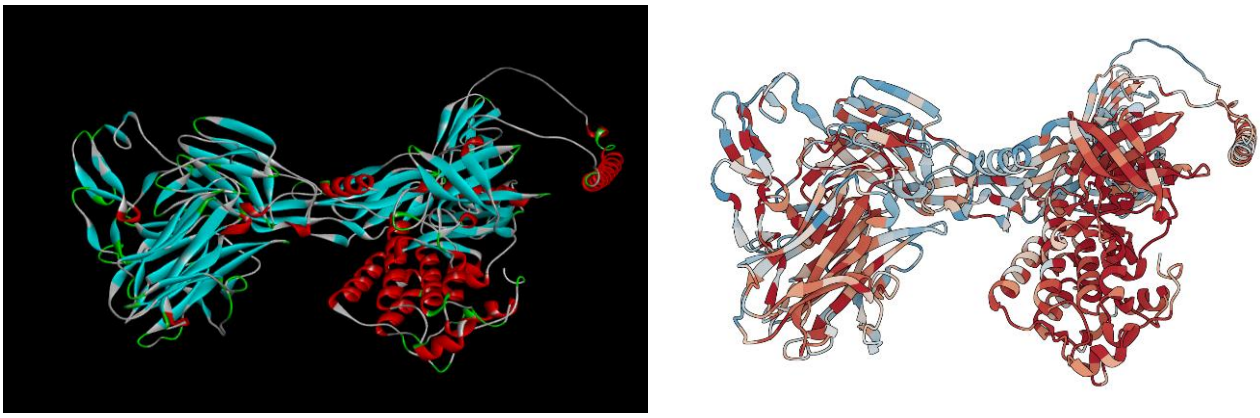
TEK

Tunica interna endothelial receptor tyrosine kinase (TEK) gene is expressed both in hematopoietic and endothelial cells (72) which regulates homeostasis. In the eye, TEK is expressed in the endothelial cells of Schlemm's canal and collector channels to regulate the flow of aqueous humor (73). Its structure is comprised of an extracellular domain having 2 immunoglobulin-like loops separated by 3 epidermal growth factor-like repeats that are connected to 3 fibronectin type III-like repeats. When TEK was knocked out from the mice model, it disturbed the homeostasis in the eye and significantly increased the intraocular pressure. This increase in IOP contributed to eye defects like PCG. TEK is only responsible for autosomal dominant primary congenital glaucoma (38). TEK mutations for PCG are common in Mexican and Chinese populations (40, 42). No mutation is reported in the TEK gene in the Pakistani population for PCG. This could be due to several factors. Genetic mutations vary in different ethnicities across the world. Due to the high rate of consanguineous marriages in Pakistani population, there are less likely chances for the involvement of autosomal dominant variants of TEK genes. Targeted sequencing of major contributed genes (CYP1B1 & LTBP2: accounting for more than 50%) for PCG cases in South Asia especially Pakistan, possibly reducing the chances of TEK gene mutations among PCG patients. The structure of TEK by X-ray crystallography and the most prone regions to mutations using AlphaFold are shown in Fig. 5.

MUTATIONS IDENTIFIED IN THE PAKISTANI POPULATION

Molecular analysis of primary congenital glaucoma has been reported in research studies in Pakistan. All mutations identified for primary congenital glaucoma in these studies are in two genes CYP1B1 and LTBP2 as listed in Table I. More than 150 CYP1B1 mutations have been linked to PCG so far,

making up a significant percentage of the genetic burden in both familial and sporadic instances of PCG (26, 74). R390H is the most common mutation of CYP1B1 genes in Pakistani populations (31).



(a) (b)

Fig. 5. Structure of TEK and position of mostly reported mutations. **(a)** PDB structure of TEK determined by X-ray crystallography **(b)** Structure determined by AlphaFold for most vulnerable positions for mutations according to literature. The brown color shows the area where, most of the time, mutation occurs.

In 2014, Sheikh found eight families with the mutations p. R390H, c.868 869insC, p.E229K, and p.A115P, as well as two families in Pakistan's Sind region with the unique mutations p.G36D and p.G67-A70del (75). Rauf conducted study on 23 families from rural Pakistan in 2016 and discovered several CYP1B1 gene variations. 11 homozygous variations were found, including seven missense mutations: c.241T>A (p.Y81N), c.685G>A (p.E229K), c.1103G>A (p.R368H), c.1405C>T (p.R390H), c.1300T>C (p.W434R), c.1331G>A (p.R444Q), and both the homozygous 1 bp deletion c.1325delC (p.P442Qfs15*) and the homozygous 10bp duplication c.1200 1209dupTCATGCCACC (p.T404Sfs30*) were novel mutations (31).

Table I. Mutations identified in the genes of Primary Congenital Glaucoma in the Pakistani population

Genes	Exons	Physical location	DNA change	c. position	Amino acid change	p. position	References
CYP1B1	2	Chr2:38302494_3830 2494	Nonsense	c.37C>T; c.38T>A	PTC	p.L13*	(30)
CYP1B1	2	Chr2:38302425	Missense	c.107G>A	G>D	p.G36D	(75)
CYP1B1	2,3	Chr2:38302423, Chr2:38298394	Compound heterozygous mutation	c.109C>T, c.1103G>A	PTC	p.Q37*	(31)
CYP1B1	2	Chr2:38302350	Missense	c.182G>A	G>E	p.G61E	(83)
CYP1B1	2	Chr2:38302323_3830 2333	Deletion	c.198_209del12	PTC	p.G67-A70del	(75)
CYP1B1	2	Chr2:38302291	Missense	c.241T>A	Y>N	p.Y81N	(31)
CYP1B1	2	Chr2:38302284_3830 2284	Deletion	c.247del	PTC	p.D83Tfs*12	(80)
CYP1B1	2	Chr2:38302245_3830 2246	Duplication	c.287dupT	PTC	p.L97Afs*12 7	(80)
CYP1B1	2	Chr2:38302177	Polymorphism	c.355G>T	A>S	p.A119S	(81)
CYP1B1	2	Chr2:38302075, Chr2:38302016	Compound Heterozygous mutation	c.457C>G, c.516C>A	R>G, S>R	p.R153G, p.S172R	(80)
CYP1B1	2	Chr2:38301990	Missense	c.542T>A	L>Q	p.(L181Q)	(78)
CYP1B1	2	Chr2:38301902_3830 1903	Duplication	c. 629dupT	PTC	p.G211Rfs*1 3	(80)
CYP1B1	2	Chr2:38301869_3830 1870	Duplication	c.662dupT	PTC	p.R222Pfs*2	(80)
CYP1B1	2	Chr2:38301847	Missense	c.685G>A	E>K	p.E229K	(75, 81, 83)
CYP1B1	2	Chr2:38301810	Missense	c.722T>A	V>E	p.V241E	(80)
CYP1B1	2	Chr2:38301800	Missense	c.732G>A	M>I	p.M244I	(80)
CYP1B1	2	Chr2:38301795_3830	Insertion	c.736_737insT	PTC	p.W246Lfs8	(31, 75)

		1796				1*	
CYP1B1	2	Chr2:38301792	Missense	c.740T>A	L>Q	p.L247Q	(80)
CYP1B1	2	Chr2:38301786	Missense	c.746G>C	A>P	p.A115P	(75)
CYP1B1	2	Chr2:38301773_3830 1774	Insertion	c.758_759insA	PTC	p.V254Gfs* 73	(80)
CYP1B1	2	Chr2:38301742_3830 1743	Duplication	c.789dupA	PTC	p.L264Afs*6 3	(80)
CYP1B1	2	Chr2:38301663_3830 1664	Duplication	c.868dupC	PTC	p.R290Pfs*3 7	(75, 78, 80)
CYP1B1	3	Chr2:38298449, Chr2:38298407	Compound heterozygous mutation	c.1048C>A; c.1090G>A	P>T, V>M	p.P350T, p.V364M	(30)
CYP1B1	3	Chr2:38298434	Non-sense	c.1063C>T	PTC	p.R355*	(83)
CYP1B1	3	Chr2:38298394	Missense	c.1103G>A	R>H	p.R368H	(31, 83)
CYP1B1	3	Chr2:38298329	Missense	c.1168C>T	R>C	p.R390C	(78)
CYP1B1	3	Chr2:38298328	Missense	c.1169G>A	R>H	p.R390H	(31, 75, 78, 82-85)
CYP1B1	3	Chr2:38298310	Missense	c.1187C>T	P>L	p.P396L	(82)
CYP1B1	3	Chr2:38298287_3829 8288	Insertion	c.1209InsTCATG CCACC	PTC	p.T404Sfs*3 0	(31, 78)
CYP1B1	3	Chr2:38298234	Missense	c.1263T>A	F>L	p.F421L	(80)
CYP1B1	3	Chr2:38298203	Missense	c.1294G>C	L>V	p. L432V	(85)
CYP1B1	3	Chr2:38298197	Missense	c.1300T>C	W>R	p.W434R	(31, 86)
CYP1B1	3	Chr2:38298187	Missense	c.1310C>T	P>L	p.P437L	(78)
CYP1B1	3	Chr2:38298171_3829 8171	Deletion	c.1325delC	PTC	p.P442Efs*1 5	(31, 78)
CYP1B1	3	Chr2:38298166	Missense	c.1331G>A	R>Q	p.R444Q	(31)
CYP1B1	3	Chr2:38298139	Missense	c.1358A>G	N>S	p. N453S	(85)
CYP1B1	3	Chr2:38298092	Missense	c.1405C>T	R>W	p.R469W	(31)
CYP1B1	3	Chr2:38298061	Missense	c.1436A>G	Q>R	p.Q479R	(78)
LTBP2	1	Chr14:75078317	Nonsense	c.331C>T	PTC	p.Q111*	(33)
LTBP2	1	chr14:75078235_750 78235	Deletion	c.412delG	PTC	p.A138PfsX 278	(33)
LTBP2	4	Chr14:75022332	Nonsense	c.895C>T	PTC	p.R299*	(33)
LTBP2	6	Chr14:75019033_750 19045	Deletion	c.1243-1256 del	PTC	p.E415RfsX 596	(33)
LTBP2	7	Chr14:75017767	Synonymous splice site mutation	c.1686G>A	-	p.Q562Q	(68)
LTBP2	8	Chr14:75016592_750 16592	De Novo mutation	c.1762_1763del	PTC	p.L588Vfs*1 4	(68)
LTBP2	16	Chr14:74989621	Canonical splice site mutation	c.2531- 2A>C	-	-	(68)
LTBP2	19	Chr14:74977948	Missense	c.3028G>A	D>N	p.D1010N	(63)
LTBP2	23	Chr14:74975631_749 75631	Deletion	c.3427delC	PTC	p.Q1143Rfs* 35	(63)
LTBP2	27	Chr14:74973402_749 73403	Insertion	c.4031_4032insA	PTC	p.D1345Gfs *6	(65)
LTBP2	34	Chr14:74969592	Missense	c.4934G>A	R>E	p.R1645E	(65)
LTBP2	35	Chr14:74968194	Missense	c.5270G>A	C>Y	p.C1757Y	(63)

Chr, Chromosome; del, Deletion; *, Deletion, dup, Duplication; ins, Insertion; fs, Frameshift, PTC, Pre-Termination Codon; >, change in amino acid

Another study found that the Pakistani population had reported already known mutations c.1169G>A (p.R390H), c.1311G>A (p.P437L), and two novel variants, c.37C>T and c.38T>A (p.L13*), which are homozygous nonsense variants caused by homozygous transition and transversion on two adjacent bases, and a compound heterozygous variants c.1048 C>A (p.P350T) & c.1090G>A(p.V364M) (30). p.P437L

was already reported in the Turkish and Brazilian populations in the homozygous state in PCG patients (76, 77).

In 2019, Rasheed 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 in *CYP1B1* (78). One novel mutation (c.1551_1551delA), chr2:38297946_38297946delT, and one known (c.1347T>C), chr2:38298150A>G were found in two families (79). In a group of eighteen families, mutations in eleven families were present in the CYP1B1 gene (80). Two missense mutations (c.355 G/T p.A119S and c.685G/A p.E229K) were found in 94% of PCG patients involved in this study (81). One novel mutation (c.1187C>T, p.Pro396Leu) and one missense mutation (c.1169G>A, p.Arg390His) have been reported in two different families of the Sind region of Pakistan (82).

Null mutations were identified in four consanguineous families of Pakistani origin in the LTBP2 gene as in Table I (33). A novel missense mutation (c.4934G>A; p.Arg1645Glu) and a novel frameshift mutation (c.4031_4032insA; p.Asp1345Glyfs*6) were discovered in two families through whole exome sequencing of LTBP2 (65). Three new pathogenic variants, c.3028G>A (p.Asp1010Asn), c.3427delC (p.Gln1143Argfs*35), and c.5270G>A (p.Cys1757Tyr), were discovered by bidirectional Sanger sequencing of LTBP2 and effectively isolated in three families (63). A new de novo frameshift mutation c.1762_1763del; p.(Leu588Valfs*14), a splice site mutation c.2531-2A>C, and a splice donor site c.1686G>A; p. (Gln562Gln) mutation were reported in three different families that are affecting the splice site region of LTPB2 (68).

CONCLUSION

CYP1B1 is the major role player in the primary congenital glaucoma among the Pakistani population. Two factors that are involved in the increased risk of primary congenital glaucoma are family history and consanguineous marriages. The most commonly reported mutation in the CYP1B1 gene is p.R390H in the majority of consanguineous families. The direct pathway of the CYP1B1 gene in ocular tissue development is unknown. This gap should be filled by more research work and animal model studies for PCG in the future. TEK gene mutations are reported in China and other countries and are associated with the pathogenicity of PCG in affected individuals. Next-generation sequencing and Whole genome/exome sequencing can pave the way for exploring other genes to know mutations in unsolved families. Moreover, Functional studies provide information on how CYP1B1, LTBP2, and TEK genes are involved in eye development. It will flourish a ground to make a diagnostic kit for the early detection of PCG in the Pakistani population and this collection of pathogenic variants acts as a start point for translational and diagnostic research.

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Conflict of interest:

There is no conflict of interest among authors regarding this review article.

References:

1. Sarfarazi MJHmg. Recent advances in molecular genetics of glaucomas. 1997;6(10):1667-77.
2. Schwartz M, Yoles EJCoio. Neuroprotection: a new treatment modality for glaucoma? 2000;11(2):107-11.
3. Firasat S, Riazuddin SA, Hejtmancik JF, Riazuddin SJMv. Primary congenital glaucoma localizes to chromosome 14q24. 2-24.3 in two consanguineous Pakistani families. 2008;14:1659.
4. Kaeslin MA, Killer HE, Fuhrer CA, Zeleny N, Huber AR, Neutzner AJPO. Changes to the aqueous humor proteome during glaucoma. 2016;11(10):e0165314.
5. Quigley HA, Addicks EM, Green WR, Maumenee AJAoo. Optic nerve damage in human glaucoma: II. The site of injury and susceptibility to damage. 1981;99(4):635-49.
6. Fechtner RD, Weinreb RNJSoo. Mechanisms of optic nerve damage in primary open angle glaucoma. 1994;39(1):23-42.

7. Burgoyne CF, Downs JC, Bellezza AJ, Suh J-KF, Hart RTJPir, research e. The optic nerve head as a biomechanical structure: a new paradigm for understanding the role of IOP-related stress and strain in the pathophysiology of glaucomatous optic nerve head damage. 2005;24(1):39-73.
8. Westerlund E. Clinical and genetic studies on the primary glaucoma diseases: Arnold Busck; 1947.
9. Anderson JR, Parsons JH. Hydrophthalmia or congenital glaucoma: Cambridge University Press; 2013.
10. Strouthidis NG, Papadopoulos MJCOR. Clinical evaluation of glaucoma in children. 2013;1:106-12.
11. Kwon YH, Fingert JH, Kuehn MH, Alward WLJNEJoM. Primary open-angle glaucoma. 2009;360(11):1113-24.
12. Wright C, Tawfik MA, Waisbourd M, Katz LJJAo. Primary angle-closure glaucoma: an update. 2016;94(3):217-25.
13. ALLEN L, Burian HM, Braley AEJAAoO. A New Concept of the Development of theAnterior Chamber Angle: Its Relationship to Developmental Glaucoma and Other Structural Anomalies. 1955;53(6):783-98.
14. Maumenee AEJTotAOS. The pathogenesis of congenital glaucoma: a new theory. 1958;56:507.
15. Kupfer C, Kaiser-Kupfer MIJAjoo. Observations on the development of the anterior chamber angle with reference to the pathogenesis of congenital glaucomas. 1979;88(3 Pt 1):424-6.
16. Anderson DJTotAOS. The development of the trabecular meshwork and its abnormality in primary infantile glaucoma. 1981;79:458.
17. Rulli E, Quaranta L, Riva I, Poli D, Hollander L, Galli F. Visual field loss and vision-related quality of life in the Italian Primary Open Angle Glaucoma Study. 2018;8(1):1-12.
18. Faiq M, Sharma R, Dada R, Mohanty K, Saluja D, Dada TJJocgp. Genetic, biochemical and clinical insights into primary congenital glaucoma. 2013;7(2):66.
19. Badawi AH, Al-Muhaylib AA, Al Owaifeer AM, Al-Essa RS, Al-Shahwan SAJSJoO. Primary congenital glaucoma: An updated review. 2019;33(4):382-8.
20. Tamçelik N, Atalay E, Bolukbasi S, Çapar O, Ozkok AJIJoO. Demographic features of subjects with congenital glaucoma. 2014;62(5):565.
21. Ava S, Demirtaş AA, Karahan M, Erdem S, Oral D, Keklikçi UJIO. Genetic analysis of patients with primary congenital glaucoma. 2021;41(7):2565-74.
22. Reis LM, Tyler RC, Weh E, Hendee KE, Schilter KF, Phillips III JA. Whole exome sequencing identifies multiple diagnoses in congenital glaucoma with systemic anomalies. 2016;90(4):378-82.
23. Chen H, Howald WN, Juchau MRJDM, Disposition. Biosynthesis of all-trans-retinoic acid from all-trans-retinol: catalysis of all-trans-retinol oxidation by human P-450 cytochromes. 2000;28(3):315-22.
24. Sarfarazi M, Stoilov IJE. Molecular genetics of primary congenital glaucoma. 2000;14(3):422-8.
25. Zhao Y, Sorenson CM, Sheibani NJJoo, research v. Cytochrome P450 1B1 and primary congenital glaucoma. 2015;10(1):60.
26. Li N, Zhou Y, Du L, Wei M, Chen XJEer. Overview of Cytochrome P450 1B1 gene mutations in patients with primary congenital glaucoma. 2011;93(5):572-9.
27. Lewis CJ, Hedberg-Buenz A, DeLuca AP, Stone EM, Alward WL, Fingert JHJHmg. Primary congenital and developmental glaucomas. 2017;26(R1):R28-R36.
28. Pan Y, Iwata TJC. Exploring the Genetic Landscape of Childhood Glaucoma. 2024;11(4):454.
29. Stoilov I, Akarsu AN, Sarfarazi MJHmg. Identification of three different truncating mutations in cytochrome P4501B1 (CYP1B1) as the principal cause of primary congenital glaucoma (Buphthalmos) in families linked to the GLC3A locus on chromosome 2p21. 1997;6(4):641-7.
30. Waryah YM, Iqbal M, Sheikh SA, Baig MA, Narsani AK, Atif M, et al. Two novel variants in CYP1B1 gene: a major contributor of autosomal recessive primary congenital glaucoma with allelic heterogeneity in Pakistani patients. 2019;12(1):8.
31. Rauf B, Irum B, Kabir F, Firasat S, Naeem MA, Khan SN. A spectrum of CYP1B1 mutations associated with primary congenital glaucoma in families of Pakistani descent. 2016;3(1):1-4.
32. Chitsazian F, Tusi BK, Elahi E, Saroei HA, Sanati MH, Yazdani S. CYP1B1 mutation profile of Iranian primary congenital glaucoma patients and associated haplotypes. 2007;9(3):382-93.
33. Ali M, McKibbin M, Booth A, Parry DA, Jain P, Riazuddin SA. Null mutations in LTBP2 cause primary congenital glaucoma. 2009;84(5):664-71.
34. Narooie-Nejad M, Paylakhi SH, Shojaee S, Fazlali Z, Rezaei Kanavi M, Nilforushan N, et al. Loss of function mutations in the gene encoding latent transforming growth factor beta binding protein 2, LTBP2, cause primary congenital glaucoma. 2009;18(20):3969-77.

35. Lim S-H, Tran-Viet K-N, Yanovitch TL, Freedman SF, Klemm T, Call W. CYP1B1, MYOC, and LTBP2 mutations in primary congenital glaucoma patients in the United States. 2013;155(3):508-17. e5.
36. Mohanty K, Tanwar M, Dada R, Dada TJMv. Screening of the LTBP2 gene in a north Indian population with primary congenital glaucoma. 2013;19:78.
37. Chen X, Chen Y, Fan BJ, Xia M, Wang L, Sun XJMV. Screening of the LTBP2 gene in 214 Chinese sporadic CYP1B1-negative patients with primary congenital glaucoma. 2016;22:528.
38. Souma T, Tompson SW, Thomson BR, Siggs OM, Kizhatil K, Yamaguchi S. Angiopoietin receptor TEK mutations underlie primary congenital glaucoma with variable expressivity. 2016;126(7):2575-87.
39. Whisenhunt KN, Tompson SW, Bradfield YS, Huang SJ, Stangel N, Higuchi EC. A missense mutation in the intracellular protein kinase domain of TEK causes autosomal dominant PCG and non-ocular features. 2017;58(8):2121-.
40. Qiao Y, Chen Y, Tan C, Sun X, Chen X, Chen JJFiG. Screening and functional analysis of TEK mutations in Chinese children with primary congenital glaucoma. 2021;12:764509.
41. Carstens N, Goolam S, Hulley M, Brandenburg J-T, Ramsay M, Williams SEIJE. Exome-based mutation screening in South African children with primary congenital glaucoma. 2023;37(2):362-8.
42. Chacon-Camacho OF, Ordaz-Robles T, Cid-García MA, Hofmann-Blancas ME, Ledesma-Gil J, García-Huerta MM, et al. TEK gene-related primary congenital glaucoma: Phenotypic features and mutational spectrum in a Mexican cohort of 10 unrelated families. 2024:e63716.
43. Muskhelishvili L, Thompson PA, Kusewitt DF, Wang C, Kadlubar FFJJoH, Cytochemistry. In situ hybridization and immunohistochemical analysis of cytochrome P450 1B1 expression in human normal tissues. 2001;49(2):229-36.
44. Bejjani BA, Stockton DW, Lewis RA, Tomey KF, Dueker DK, Jabak M. Multiple CYP1B1 mutations and incomplete penetrance in an inbred population segregating primary congenital glaucoma suggest frequent de novo events and a dominant modifier locus. 2000;9(3):367-74.
45. Doshi M, Marcus C, Bejjani BA, Edward DPJEer. Immunolocalization of CYP1B1 in normal, human, fetal and adult eyes. 2006;82(1):24-32.
46. Yamazaki S, Sato K, Suhara K, Sakaguchi M, Mihara K, Omura TJTJoB. Importance of the proline-rich region following signal-anchor sequence in the formation of correct conformation of microsomal cytochrome P-450s. 1993;114(5):652-7.
47. Swindell EC, Eichele GJB. Retinoid metabolizing enzymes in development. 1999;10(2-3):85-9.
48. Banerjee A, Chakraborty S, Chakraborty A, Chakrabarti S, Ray KJPO. Functional and structural analyses of CYP1B1 variants linked to congenital and adult-onset glaucoma to investigate the molecular basis of these diseases. 2016;11(5):e0156252.
49. Faiq MA, Dada R, Qadri R, Dada TJJocgp. CYP1B1-mediated pathobiology of primary congenital glaucoma. 2015;9(3):77.
50. Ling C, Zhang D, Zhang J, Sun H, Du Q, Li XJE. Updates on the molecular genetics of primary congenital glaucoma. 2020;20(2):968-77.
51. Zavarzadeh PG, Shalbafan B, Abdi FJJoO, Sciences O. Primary Congenital Glaucoma: Detecting Novel Disease-Causing Variants Using Whole-Exome Sequencing and Pathway Analysis. 2020;4(1):43-59.
52. Shen X, Koga T, Park B-C, SundarRaj N, Yue BYJJoBC. Rho GTPase and cAMP/protein kinase A signaling mediates myocilin-induced alterations in cultured human trabecular meshwork cells. 2008;283(1):603-12.
53. Chen W, Yang X, Fang J, Zhang Y, Zhu W, Yang XJFip. Rho-associated protein kinase inhibitor treatment promotes proliferation and phagocytosis in trabecular meshwork cells. 2020;11:302.
54. Zhao Y, Wang S, Sorenson CM, Teixeira L, Dubielzig RR, Peters DM. Cyp1b1 mediates periostin regulation of trabecular meshwork development by suppression of oxidative stress. 2013;33(21):4225-40.
55. Falero-Perez J, Song Y-S, Sorenson CM, Sheibani NJTic, biology m. CYP1B1: A key regulator of redox homeostasis. 2018;13:27.
56. Schwartzman ML, Balazy M, Masferrer J, Abraham NG, McGiff JC, Murphy RCJPotNAoS. 12 (R)-hydroxyicosatetraenoic acid: a cytochrome-P450-dependent arachidonate metabolite that inhibits Na+, K+-ATPase in the cornea. 1987;84(22):8125-9.
57. Safari I, Suri F, Haji-Seyed-Javadi R, Yazdani S, Elahi EJOR. The p. Gly61Glu mutation in CYP1B1 affects the extracellular matrix in glaucoma patients. 2016;56(2):98-103.
58. Xia Q, Zhang D, Zhuang Y, Dai Y, Jia H, Du Q,. Animal Model Contributions to Primary Congenital Glaucoma. 2022;2022.

59. Chambers D, Wilson L, Maden M, Lumsden A. RALDH-independent generation of retinoic acid during vertebrate embryogenesis by CYP1B1. 2007.
60. Williams AL, Eason J, Chawla B, Bohnsack BLJIo, science v. Cyp1b1 regulates ocular fissure closure through a retinoic acid–independent pathway. 2017;58(2):1084-97.
61. Williams AL, Bohnsack BLJBDRPCETR. Neural crest derivatives in ocular development: discerning the eye of the storm. 2015;105(2):87-95.
62. Shipley JM, Mecham RP, Maus E, Bonadio J, Rosenbloom J, McCarthy RT. Developmental expression of latent transforming growth factor β binding protein 2 and its requirement early in mouse development. 2000.
63. Rauf B, Irum B, Khan SY, Kabir F, Naeem MA, Riazuddin S. Novel mutations in LTBP2 identified in familial cases of primary congenital glaucoma. 2020;26:14.
64. Azmanov DN, Dimitrova S, Florez L, Cherninkova S, Draganov D, Morar B. LTBP2 and CYP1B1 mutations and associated ocular phenotypes in the Roma/Gypsy founder population. 2011;19(3):326-33.
65. Micheal S, Siddiqui SN, Zafar SN, Iqbal A, Khan MI, den Hollander AIJPO. Identification of novel variants in LTBP2 and PXDN using whole-exome sequencing in developmental and congenital glaucoma. 2016;11(7):e0159259.
66. Horiguchi M, Ota M, Rifkin DBJTjob. Matrix control of transforming growth factor-β function. 2012;152(4):321-9.
67. Kielty CM, Sherratt MJ, Marson A, Baldock CJAipc. Fibrillin microfibrils. 2005;70:405-36.
68. Saleem RS, Khan MI, Irshad S, Siddiqui SN, Micheal S. Identification of de Novo and Novel Mutations in LTBP2 in Pakistani Families with Inherited Primary Congenital Glaucoma. 2024.
69. Hirani R, Hanssen E, Gibson MAJMB. LTBP-2 specifically interacts with the amino-terminal region of fibrillin-1 and competes with LTBP-1 for binding to this microfibrillar protein. 2007;26(4):213-23.
70. Inoue T, Ohbayashi T, Fujikawa Y, Yoshida H, Akama TO, Noda K. Latent TGF-β binding protein-2 is essential for the development of ciliary zonule microfibrils. 2014;23(21):5672-82.
71. Désir J, Sznajer Y, Depasse F, Roulez F, Schrooyen M, Meire F. LTBP2 null mutations in an autosomal recessive ocular syndrome with megalocornea, spherophakia, and secondary glaucoma. 2010;18(7):761-7.
72. Wada M, Ebihara Y, Ma F, Yagasaki H, Ito M, Takahashi T. Tunica interna endothelial cell kinase expression and hematopoietic and angiogenic potentials in cord blood CD34+ cells. 2003;77:245-52.
73. van Zyl T, Yan W, McAdams A, Peng Y-R, Shekhar K, Regev A. Cell atlas of aqueous humor outflow pathways in eyes of humans and four model species provides insight into glaucoma pathogenesis. 2020;117(19):10339-49.
74. Stenson PD, Mort M, Ball EV, Shaw K, Phillips AD, Cooper DNJHg. The Human Gene Mutation Database: building a comprehensive mutation repository for clinical and molecular genetics, diagnostic testing and personalized genomic medicine. 2014;133(1):1-9.
75. Sheikh SA, Waryah AM, Narsani AK, Shaikh H, Gilal IA, Shah K. Mutational spectrum of the CYP1B1 gene in Pakistani patients with primary congenital glaucoma: novel variants and genotype-phenotype correlations. 2014;20:991.
76. Stoilov I, Akarsu AN, Alozie I, Child A, Barsoum-Homsy M, Turacli ME. Sequence analysis and homology modeling suggest that primary congenital glaucoma on 2p21 results from mutations disrupting either the hinge region or the conserved core structures of cytochrome P4501B1. 1998;62(3):573-84.
77. Della Paolera M, de Vasconcellos JPC, Umbelino CC, Kasahara N, Rocha MN, Richeti F. CYP1B1 gene analysis in primary congenital glaucoma Brazilian patients: novel mutations and association with poor prognosis. 2010;19(3):176-82.
78. Rashid M, Yousaf S, Sheikh SA, Sajid Z, Shabbir AS, Kausar T. Identities and frequencies of variants in CYP1B1 causing primary congenital glaucoma in Pakistan. 2019;25:144.
79. Shan T, Gul B, Otho SA, Manzoor S, Noureen A, Rind KH. Mutational Analysis of CYP1B1 gene in families with Primary Congenital glaucoma. 2021;20(5):5285-92.
80. Tehreem R, Arooj A, Siddiqui SN, Naz S, Afshan K, Firasat SJPo. Mutation screening of the CYP1B1 gene reveals thirteen novel disease-causing variants in consanguineous Pakistani families causing primary congenital glaucoma. 2022;17(9):e0274335.
81. Zahid T, Khan MU, Zulfiqar A, Jawad F, Saleem A, Khan ARJPJoMS. Investigation of mutational spectrum in cytochrome P4501B1 (CYP1B1) as the principal cause of primary congenital glaucoma. 2023;39(2):409.

82. Surhio WA, Khidri FF, Haroon MI, Mehmood S, Waryah YMJJoLUoM, Sciences H. Mutation Screening of the CYP1B1 Gene Reveals Novel and Recurrent Pathogenic Variants in Pakistani Primary Congenital Glaucoma Patients. 2024;23(02):140-5.
83. Afzal R, Firasat S, Kaul H, Ahmed B, Siddiqui SN, Zafar S. Mutational analysis of the CYP1B1 gene in Pakistani primary congenital glaucoma patients: Identification of four known and a novel causative variant at the 3′ splice acceptor site of intron 2. 2019;59(5):152-61.
84. Micheal S, Ayub H, Zafar SN, Bakker B, Ali M, Akhtar F. Identification of novel CYP1B 1 gene mutations in patients with primary congenital and primary open-angle glaucoma. 2015;43(1):31-9.
85. Khan MU, Rehman R, Kaul H, Mahmood S, Ammar AJAiLS. Mutational analysis of CYP1B1 gene in Pakistani pediatric patients affected with Primary Congenital Glaucoma. 2019;7(1):32-7.
86. Firasat S, Kaul H, Ashfaq UA, Idrees SJIo. In silico analysis of five missense mutations in CYP1B1 gene in Pakistani families affected with primary congenital glaucoma. 2018;38:807-14.