Glaucoma Genetics in Pakistan

18

Humaira Ayub, Maleeha Azam, Shazia Micheal, Muhammad Imran Khan, Farah Akhtar, Chiea Chuen Khor, Tin Aung, Anneke den Hollander, and Raheel Qamar

Abstract

Glaucoma, a multifactorial ocular disease, is clinically and genetically heterogeneous. It is the second leading cause of blindness in elderly population worldwide. Because of the complex nature of glaucoma, the genetic spectrum has not been established globally. In Pakistan, both the familial and the sporadic forms of the disease are common, which is attributed to higher percentage of consanguinity in the Pakistani population. Till the year 2008, there were no reports from Pakistan about the genetic factors causing glaucoma. In order to identify the glaucoma genetic spectrum in the Pakistani population, we genetically screened individuals with glaucoma that included the common clinical subclasses; primary congenital glaucoma (PCG), primary open angle glaucoma (POAG), primary angle closure glaucoma (PACG), and pseudoexfoliation glaucoma (PEXG). We conducted linkage analysis of the glaucoma families,

H. Ayub
Department of Biomedical Sciences, Pak-Austria Fachhochschule: Institute of Applied Sciences and Technology Mang, Haripur, Pakistan

M. Azam
Department of Biosciences, COMSATS University Islamabad, Islamabad Campus, Islamabad, Pakistan

S. Micheal
Department of Ophthalmology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

M. Imran Khan
Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

F. Akhtar
Al-Shifa Trust Eye Hospital, Rawalpindi, Pakistan

C. C. Khor
Division of Human Genetics, Genome Institute of Singapore, Singapore, Singapore
e-mail: khorcc@gis.a-star.edu.sg

T. Aung
Singapore Eye Research Institute (SERI) & Singapore National Eye Center (SNEC), Singapore, Singapore

Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore
e-mail: aung.tin@singhealth.com.sg

A. den Hollander
Department of Ophthalmology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands
e-mail: A.denHollander@ohk.umcn.nl

R. Qamar (✉)
Department of Biosciences, COMSATS University Islamabad, Islamabad Campus, Islamabad, Pakistan

ICESCO, Rabat, Morocco

Pakistan Academy of Sciences, Islamabad, Pakistan

G. Prakash, T. Iwata (eds.), *Advances in Vision Research, Volume III*, Essentials in Ophthalmology,
https://doi.org/10.1007/978-981-15-9184-6_18

case-control association analysis of the sporadic glaucoma cases using previously reported single nucleotide polymorphisms (SNPs), and also carried out genome wide association studies (GWAS). These studies have allowed us to discover novel glaucoma causing genes and risk-associated SNPs in the Pakistani population. The identification of novel glaucoma genes reveals novel molecular mechanisms involved in glaucoma pathogenesis. However, the clinical heterogeneity in the Pakistani glaucoma population suggests the need for further exploration of the molecular/genetic causes of the disease.

Keywords

Glaucoma · GWAS · SNPs · *CYP1B1* · Novel genes/loci

18.1 Introduction

Glaucoma is a group of neurodegenerative ocular diseases, which is caused by optic nerve damage either in one eye or both, leading to visual field defect and eventually blindness.

Glaucoma is a multifactorial disease, caused by interplay of genetic and environmental factors. The complex nature of the disease is either due to heterogeneity where different genes result in the same clinical subtype or the same genes lead to variable clinical conditions. Phenocopies have also been observed that are caused by environmental factors. The presence of modifier element also makes the condition complex where mutation carriers remain asymptomatic. Deviation from the Mendelian mode of inheritance is common in glaucoma [1–4]. Due to all these different factors analyzing glaucoma genetically is difficult, however, familial clustering as well as association studies have been helpful in identification of pathogenic mutations as well as rare and common polymorphic genetic variants that have shown higher percentages in affected individuals as compared to healthy (unaffected) control population [5]. Having a multifaceted etiology, certain genes are pertinent to glaucoma progression globally as well as in a population-specific manner. In Pakistan, the higher prevalence is not only due to the lack of awareness and management of the disease but also the clinical heterogeneity where different subclasses have familial form and sporadic occurrence, which affect people of all ages. Our genetic screening of glaucoma, which included primary congenital glaucoma (PCG), primary open angle glaucoma (POAG), primary angle closure glaucoma (PACG), and pseudoexfoliation glaucoma (PEXG), involved replication studies, which revealed glaucoma-associated single nucleotide polymorphisms (SNPs) in the Pakistani population, exome sequencing resulted in novel gene identification. While global screening of glaucoma including the Pakistani cohort, led to the discovery of glaucoma-associated novel loci. All the studies performed until now have indicated genetics as one of the major components involved in familial and sporadic glaucoma onset in Pakistan.

18.2 Global Perspective

Glaucoma displays variable occurrence rate among different populations worldwide, similarly its genetic etiology exhibits heterogeneity and therefore remains largely unknown not only globally but also in different populations including Pakistan. Glaucoma is estimated to affect worldwide 79.6 million by 2020 [6] and 111.8 million by 2040 [7], three-fourth of whom will suffer from POAG [6]. Females are more prone to develop the disease, encompassing 70% of PACG patients, 55% of POAG, and 59% of other types of glaucoma. The ethnicity differences reveal that Africans have the highest occurrence rate, where POAG is observed to be more common, followed by Asians, where the Chinese population has a higher incidence of PACG patients, while normal tension glaucoma (NTG) subtype is more common among the Japanese [8].

Previously familial studies resulted in identification of 18 glaucoma loci (GLC1A to GLC1N,

GLC3A to GLC3D). The mutated genes included optineurin (*OPTN*), myocillin (*MYOC*), neurotrophin 4 (*NTF4*), and WD repeat domain 36 (*WDR36*) [9, 10]. Among the subclinical classes, PCG is more common in infants, usually occurring in sporadic manner in outbred populations, however, it is inherited recessively in inbred population [11, 12]. To date, four loci (GLC3A, GLC3B, GLC3C, and GLC3D) have been linked to PCG, with two identified genes GLC3A (*CYP1B1*) and GLC3D (Latent transforming growth factor beta binding protein; *LTBP2*) [13, 14]. Among the reported genes, cytochrome P450 (*CYP1B1*) has been found to be associated with PCG in different populations globally [9]. Through GWAS and case-control association studies in different cohorts, around 20 genes have been reported for POAG [9], while 9 genes have been found to be associated with PACG [15, 16]. More than 70 point mutations in *MYOC* have been found to be associated with POAG (predominantly 3–5% being associated with juvenile open angle glaucoma (JOAG)) [17] worldwide. Despite the identification of a number of genes, the complete genetic etiology of glaucoma remains undefined.

18.3 Epidemiology

A comprehensive report on glaucoma prevalence in Pakistan is still lacking, though small hospital-based studies have been conducted they do not provide a complete epidemiological overview of the Pakistani glaucoma patients. Therefore, there remains a gap in the determination of epidemiological basis of glaucoma of the Pakistani population. World health organization (WHO) national survey of blindness conducted between 1987 and 1990 showed that the prevalence of blindness varied from 2.0 to 4.3% in the Pakistani population, where glaucoma was found to be responsible for 3.9% blindness cases [18], which rose to 7.1% by the year 2007 [19]. However, another study has shown that 1.8 million people in Pakistan are affected by glaucoma among them one million are legally blind. Although it has a high prevalence, glaucoma is the major cause of treatable blindness in Pakistan second to cataract [19].

In Pakistan, POAG is the most frequent glaucoma subtype [20], especially in adults who are 70 years and older [19]. Moreover, PACG is more common among females and POAG among the males [21]. Congenital glaucoma accounts for up to 18% of childhood blindness and is believed to occur in 1 among 2000 births in the Middle East and 1 in 10,000 births in Western countries [22]. The higher occurrence of glaucoma in the Middle East is attributed to higher percentage of consanguineous marriages, which is even higher in South Asian countries including Pakistan [23].

18.4 Etiology

The etiology of glaucoma is complex with involvement of a combination of factors resulting in a similar pathological outcomes. The major risk factor of high prevalence of isolated primary glaucoma in Pakistan is old age [24]. Besides that, based on the recent genetic studies, the role of genetic susceptibility is becoming apparent. The major cause of familial glaucoma in Pakistan is consanguinity that results in many diseased recessive families, though dominant glaucoma families have also been identified worldwide [25], we also observed few dominant conditions in our studied cohort. Pakistan has one of the highest rates of consanguinity, because of which the ratio of PCG is higher followed by JOAG. Due to excessive inbreeding, clinical extremes have been observed in Pakistani patients. Among the environmental factors, exposure to sun also plays a role in disease manifestation as the people in the rural areas are usually farmers and are excessively exposed to sunlight. The major reason of occurrence of secondary form of glaucoma in Pakistan is use of steroid, for keratoconjuctivites, and allergies as well as self-medication for eye problems. The second most common cause of secondary glaucoma found in the Pakistani population is bilateral penetrating keratoplasty [26]. Besides, diabetes, cataract, and other diseases may also lead to secondary form of neovascular

glaucoma [27]. Due to a lack of awareness, delay in diagnosis, and treatment facilities in Pakistan, patients lose eyesight thus worsening their condition [28]. In addition, there are a number of systemic disorders called as glaucoma syndromes, which involve ocular abnormalities as a secondary feature [29]. These include Marfan's syndrome, Alfred Reiger's syndrome, Nail-patella syndrome, and Pigment Dispersion syndrome.

Though glaucoma induced blindness is preventable, numerous studies have demonstrated that access to glaucoma care facilities and noncompliance to therapy are still the major issues to be addressed in Pakistan. Several interrelated factors may contribute to noncompliance, including illiteracy in patients, self-medication, and poor socioeconomic status, which may be one of the reasons behind discontinuation of medicines resulting in progression of glaucoma to end stage. All these factors must be investigated in a glaucoma patient's noncompliance to medical treatment [30].

18.5 Experimental

In order to study the glaucoma genetic spectrum in the Pakistani population, we genetically analyzed familial and sporadic glaucoma, including the major clinical subclasses PCG, POAG, PACG, and PEXG. The genetic screening was started in 2008 and is still ongoing. In addition to that, all the glaucoma genetic findings by other groups working on Pakistani patients were also collated with our data in the current study to better understand the genetic etiology of the Pakistani population.

18.5.1 Identification of Novel Genes, Loci, and Novel Mutations in Known Genes

18.5.1.1 Homozygosity Mapping and Exome Sequencing

Glaucoma families were screened by exclusion mapping using Sanger sequencing, in these families, glaucoma was the primary cause of vision loss and also a secondary clinical feature in syndromic families. Using this technique few novel mutations in known genes were identified. The unsolved families were further analyzed by whole exome sequencing and homozygosity mapping after microarray analysis of selected family members, this resulted in identification of a few novel genes/loci (Table 18.1).

18.5.1.2 Genome Wide Association Studies

For the association studies, multiple techniques were used such as genome wide association studies (GWAS) [31, 32] that led to identification of novel loci (Table 18.2). Replication studies of selected SNPs based on their previous association with various populations were done through TaqMan/KASPAR assays, this highlighted the previously identified glaucoma associated SNPs role in the Pakistani population (Table 18.2).

18.6 Genetic Aspects

18.6.1 Familial Glaucoma Genetics in Pakistan

18.6.1.1 CYP1B1 Associated Glaucoma Families

Due to consanguinity in Pakistan, there is a frequent transmission of mutations through the generations resulting in a higher prevalence of genetic diseases. Many genes are expected to be involved in the progression of familial glaucoma in the Pakistani population, therefore the exact genetic cause remains undefined [33]. The GLC3A locus on chromosome 2 has been reported to be the most significant contributor to recessive PCG in the Pakistani population [34, 35]. *CYP1B1* is the gene that resides in the GLC3A locus and is one of the major causes of glaucoma [(PCG (34.6%) and POAG (3.3%)] in the Pakistani population (Fig. 18.1). The founder mutation p.Arg390His in *CYP1B1*, is the most frequent *CYP1B1* mutation not only in the Pakistani population (45%; Fig. 18.1) but

Table 18.1 Genes and their identified mutations causative of familial glaucoma in the Pakistani population

Gene (MIM ID)	Mutation (protein variation)	Phenotype	Chromosomal location	References
CYP1B1 (MIM: 601771)	p.Leu177Arg	PCG	2p22.2	[37]
	p.Leu487Pro	PCG		[37]
	p.Asp374Glu	PCG		[37]
	p.Arg390His[a]	PCG+POAG		[35, 38–42]
	p.Arg355*	PCG		[38, 40]
	p.Glu229Lys	POAG		[38–40]
	p.Ala288Pro	PCG		[38]
	p.Asp242Ala	PCG		[38]
	p.Arg290Profs*37	PCG		[38]
	p.Asp316Val	POAG		[38]
	p.Ala115Pro	PCG		[39]
	c.868_869insC	PCG		[39]
	p.Gly36Asp	PCG		[39]
	p.Gly67-Ala70del	PCG		[39]
	p.Trp434Arg	PCG		[35]
	p.Arg444Gln	PCG		[35]
	p.Tyr81Asn	PCG		[35]
	p.Arg368His	PCG		[35, 40, 41]
	p.Trp246Leufs81* + p.Glu299Lys	PCG		[35, 41]
	p.Pro442Glnfs15*	PCG		[35]
	p.Gln37*	PCG		[35]
	p.Arg469Trp	PCG		[35]
	p.Thr404Serfs30*	PCG		[35]
	c.1044-1G>C	PCG		[40]
	p.Gly61Asp			
	p.Pro437Leu	PCG		[42]
	p.Pro350Thr + p.Val364Met	PCG		[42]
	p.Leu13*	PCG		[42]
LTBP2 (MIM: 602091)	p.Arg299X p.Ala138Profs*278, p. Gln111X p.Glu415Argfs*596 p.Arg1645Glu p.Asp1345Glyfs*6	PCG	14q24.3	[44]
		PCG		
		PCG		
		PCG		
		PCG		[45]
		PCG		
MYOC (MIM: 601652)	p.Thr377Arg	JOAG	1q24.3	[47]
PXDN (MIM: 605158)	p.Gly1166Arg	PCG	2p25.3	[45]
PRPF8 (MIM: 607300)	p.Pro13Leu and p.Met25Thr	POAG	7q31.2	[46]
FOXC1 (MIM: 601090)	p.Ala31_Ala33del	PCG	6p25.3	[49]
PAX6 (MIM: 607108)	p.Tyr75*	Axenfeld-Rieger syndrome	11p13	[49]

(continued)

Table 18.1 (continued)

Gene (MIM ID)	Mutation (protein variation)	Phenotype	Chromosomal location	References
FBN1 (MIM: 154700)		Marfan's Syndrome	15q21.1	[50]
MYO18A (MIM: 610067)	p.Arg691Cys	JOAG	17q11.2	Ayub et al. [unpublished data]
ENOX1 (MIM: 610914)	p.Met57Ile		13q14.11	
COL9A2 (MIM: 120260)	p.Pro354Leu		1p34.2	
NCOA7 (MIM: 609752)	p.Val242Met	Late onset POAG	6q22.31-q22.32	Ayub et al. [unpublished data]
PHKG1 (MIM:172470)	p.Thr42Met	JOAG	7p11.2	Ayub et al. [unpublished data]
Novel Locus		PCG	14q24.2-24.3	[48]
Novel Locus		PCG	7q34	Ayub et al. [unpublished data]

'*' Stop codon, Under mutation section "+" indicates occurrence of two mutations in a single family, Under the phenotype section "+" indicates the coexistence of mentioned phenotypes in the family
[a]Founder mutation of Pakistani population

is also frequently reported in Saudi and South Korean populations [36]. The first report of the involvement of *CYP1B1* in glaucoma families of Pakistani origin was in 2008 with identification of three novel missense mutations (p.Leu177Arg, p.Leu487Pro, and p.Asp374Glu) in the gene [37]. Exclusion mapping that we performed in our cohort of 40 glaucoma families (12 PCG and 28 POAG) revealed one known and three novel homozygous mutations in *CYP1B1* in four PCG families (p.Arg355*, p.Ala288Pro, p.Asp242Ala, and p.Arg290Profs*37). The p.Arg390His is the most frequent mutation that we identified in our cohort [38]. In addition in a panel of POAG families, a novel heterozygous missense mutation (c.947A>T; p.Asp316Val) was identified along with a known mutation (p.Glu229Lys). The latter was also found in three other POAG families [38].

A study conducted by Sheikh et al. [39] on a panel of 20 PCG families that were screened by short tandem repeat (STR) markers spanning *CYP1B1,* revealed linkage of half of the panel (ten families) homozygously to *CYP1B1* region. Six mutations were identified in the *CYP1B1* linked families, with p.Arg390His being the most frequent mutation. Rauf et al. [35] identified two novel mutations p.W246Lfs81* and p.P442Qfs15* in *CYP1B1* in PCG families whereas nine recurrent mutations in their panel of 23 PCG families were identified where the founder p.Arg390His mutation was found to be segregating in 13/23 families. In another study by Afzal et al. [40] on a panel of 38 PCG families, ten families showed linkage to *CYP1B1* with the identification of one novel mutation (c.1044-1G>C) in the 3′ splice site in one family, while three other had recurrent mutations. Bashir et al. [41] recently identified a novel (c.736dupT, p.W246LfsX81*) and recurrent mutations in the *CYP1B1* in five out of six PCG families in their panel. In a recent study, direct sequencing of 11 PCG families for *CYP1B1* resulted in identification of mutation in seven families, this included a novel mutation p.P437L, compound heterozygous variants p.P350T and p.V364M as well as two known mutations p.R390H and p.P437L [42].

Table 18.2 Sporadic glaucoma associated novel genes and loci through genome wide association studies and replication studies

Gene	SNP ID	Glaucoma subtype	OR (95%CI) *p*-Value	References
CHAT (MIM:254210)	rs1258267	PACG	1.22 (1.58–3.98) 4.99×10^{-16}	[31]
POMP (MIM: 601952)	rs7329408	PEXG	1.13 (1.07–1.19) 1.61×10^{-5}	[32]
TMEM136 (MIM: 614465)	rs11827818	PEXG	1.15 (1.08–1.22) 4.35×10^{-6}	
AGPAT1 (MIM: 603099)	rs3130283	PEXG	1.24 (1.14–1.34) 2.27×10^{-7}	
RBMS3 (MIM: 605786)	rs12490863	PEXG	1.12 (1.05–1.20) 0.00053	
near *SEMA6A*	s10072088	PEXG	0.88(0.81–0.96) 0.0024	
ASB10 (MIM: 602432)	rs2253592	POAG	$P = 0.047$	[51]
TMCO1 (MIM: 213980)	rs4656461	POAG[a] PACG PEXG[a]	0.57(0.38–0.89) 0.003 0.52 (0.30–0.88) 0.009 0.54 (0.32–0.92) 0.01	[52]
ATOH7 (MIM: 221900)	rs1900004	PACG[a]	0.69(0.48–1.00) 0.03	[52]
CAV1 (MIM: 606721)	rs4236601	POAG	2.46 (1.01–6.24) 0.02	[52]
BIRC6 (MIM: 605638)	rs2754511	PEXG	0.42 (0.22–0.81) 0.05	[59]
XRCC1 (MIM: 617633)	rs25487	POAG	2.65(1.44–4.85), $p < 0.005$)	[53]
XPD (MIM: 278730)	rs13181	POAG	1.89 (1.23–2.9), $p = 0.005$)	[53]
LOXL1 (MIM: 177650)	rs1048661	PEXG	2.98 (1.94–4.57) 0.0001	[54]
	rs3825942	PEXG	6.83 (2.94–16.67) 0.00001	[54]
MTHFR (MIM: 181500)	rs1801133	PACG	1.09 (0.64–1.84) 0.001	[58]
NOS3 (MIM: 104300)	27bp intron 4 VNTR	POAG PACG PEXG	1.74 (1.10–2.75) 0.01 2.09 (1.23–3.55) 0.001 1.68 (1.01–2.7) 0.04	[55], Ayub et al. [unpublished data]
HSP70 (MIM: 140550)	rs1043618	POAG PACG PEXG	2.68 (1.79–4.01) 2.22 e^{-09} 1.91(1.18–3.10) 0.002 2.87 (1.75–4.71) 2.5 e^{-07}	[55], Ayub et al. [unpublished data]
COL11A1 (MIM: 228520)	rs3753841	PEXG	0.44 (0.19–1.0) 0.05	Ayub et al. [unpublished data]
GST (MIM: 138350)	M1	PEXG	20.77 (2.45–460.38) 0.001	[60]
	T1	PEXG	4.47 (1.96–10.29) 0.001	[60]

(continued)

Table 18.2 (continued)

Gene	SNP ID	Glaucoma subtype	OR (95%CI) *p*-Value	References
TNFα (MIM: 157300)	G-308A	PEXG[a]	0.24 (0.12–0.51) <0.001	[56]
MYOC (MIM: 137750)	rs74315341		197.01 $p = 0.04$	[33]
	rs879255525		199.25 $p = 0.016$	[33]
MMP1 (MIM: 226600)	rs1799750	POAG	2.14 (1.10–4.15) 0.001	[57]
MMP7 (MIM: 178990)	rs17576	PACG	1.34 (0.73–2.47) 0.35	[57]
CYP1B1 (MIM: 231300)	rs2567206	PEXG[a]	0.44 (0.25–0.77)/0.0002	Ayub et al. [unpublished data]

[a]Protective role

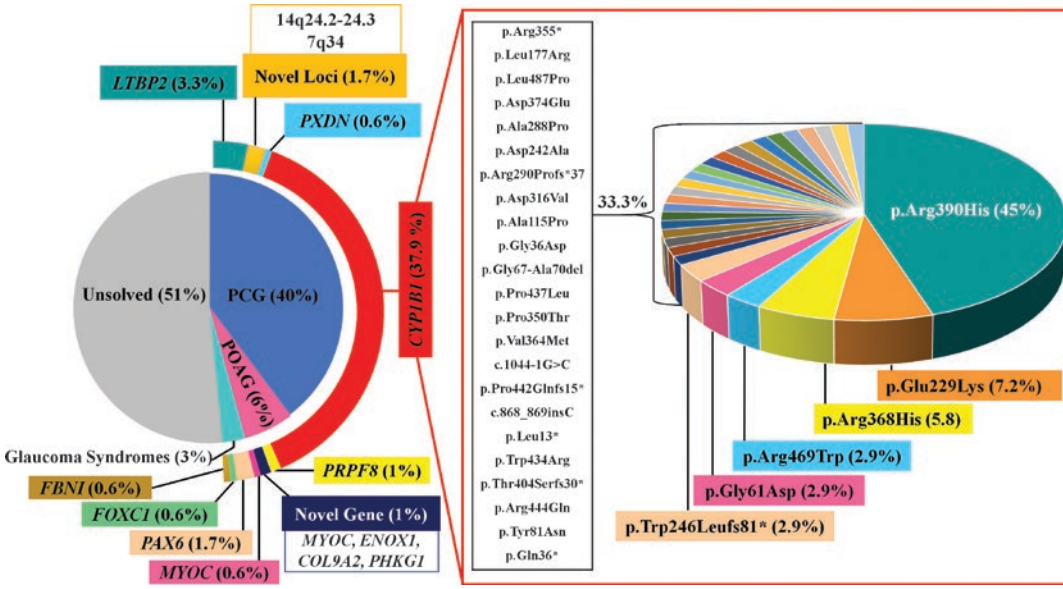

Fig. 18.1 Frequency of genes associated with PCG, POAG, and glaucoma syndromes in the Pakistani population in a cohort of 182 families. *CYP1B1* is the major cause of PCG and POAG. Frequency of the *CYP1B1* glaucoma causing mutations also represented, which accounts for 37.9% of families carrying *CYP1B1* mutations in the Pakistani Population (data compiled from studies [35, 37–42, 44–50])

Though involvement of *CYP1B1* is apparent in PCG in Pakistan, interestingly, our group identified homozygous *CYP1B1* mutation p.Arg390His in a large consanguineous family that had heterogeneous clinical presentation. The members were affected with POAG (both Juvenile and late onset) as well as PCG [38]. Similarly, a homozygous mutation c.182G>A, p.G61E in *CYP1B1* was also found to be responsible for both Juvenile onset POAG (27 years) as well as PCG in another consanguineous Pakistani family [43]. We also identified a novel heterozygous missense mutation (p.Asp316Val) in a late-onset POAG family thus extending the mutation spectrum of *CYP1B1* in Pakistani glaucoma families [38].

Data pooling from the studies done on glaucoma families of Pakistani origin revealed that since 2008 till date nearly 182 families have been genetically screened, where mutations in *CYP1B1* were found in 37.9% of the families (Fig. 18.1), most of these families were screened by direct *CYP1B1* sequencing, in the remaining families

(51%) there might be a deep intronic variant in *CYP1B1* causative of the disease or involvement of some other gene or some nongenic part. Therefore, there is a possibility of novel gene discovery in the unsolved PCG families.

18.6.2 Other Genes Involved in Familial Glaucoma

Second most frequently mutated gene (3.3%) in the PCG families was *LTBP2* (latent transforming growth factor beta binding protein, Fig. 18.1). To date, 6 mutations in *LTBP2* have been identified to cause PCG in Pakistan; Ali et al. [44] identified a homozygous nonsense mutation in exon 4 (c.895C >T; p.Arg299X), a homozygous single base pair deletion in exon 1 (c.412delG; p.Ala138Profs*278), a homozygous nonsense variant in exon 1 (c.331C>T; p.Gln111X) and a homozygous 14-base pair deletion in exon 6 (c.1243-1256 del; p.Glu415Argfs*596) [44]. In our cohort, a missense mutation (c.4934G>A; p.Arg1645Glu) and a novel frameshift mutation (c.4031_4032insA; p.Asp1345Glyfs*6) were also identified in *LTBP2* after whole exome sequencing of PCG Pakistani families [45]. Another family in our cohort was linked to *PXDN* with a novel missense mutation (c.3496G>A; p.Gly1166Arg) [45]. *PRPF8* that was previously identified to cause retinitis pigmentosa was found to be causative of POAG in Pakistani families, where two nonsynonymous variants p.Pro13Leu and p.Met25Thr were identified to be segregating with the POAG phenotype [46]. Another gene, *MYOC*, has also been reported to cause glaucoma in Pakistan with the identification of a heterozygous mutation (p.Thr377Arg) in a family with severe glaucoma phenotype [47].

The locus 14q24.2–24.3 was found segregating in two consanguineous Pakistani families in a study conducted in 2008 [48]. In another study [Ayub et al. unpublished data], homozygosity mapping revealed a novel locus 7q34 to be present homozygously in the affected members of a small PCG family. Though targeted exome sequencing was performed of the 2 MB locus, no plausible disease-associated gene was identified thus indicating the possible involvement of deep intronic mutation or nongenic region or some other gene outside of this region.

We also obtained interesting results with whole exome sequencing of POAG families [Ayub et al. unpublished]. In a large consanguineous dominant POAG family, we identified three variants in three novel genes (*MYO18A*: c.2071G>A; p.Arg691Cys, *ENOX1*: c.171C>T; p.Met57Ile, *COL9A2*: c.1061C>T; p.Pro354Leu) segregating with the disease. The presence of all the variants resulted in the early onset of the disease (discussed in clinical part). Whereas another variant (*NCOA7*: c.724C>T; p.Val242Met) segregated in a different loop of the same family, in this branch the three variants did not exist together and the affected persons had late onset of the disease. In another consanguineous Pakistani family with juvenile-onset POAG, inherited dominantly, whole exome sequencing identified a variant (c.125C>T p.Thr42Met) in the *PHKG1* to be segregating heterozygously [Ayub et al. unpublished data].

18.6.3 Genetics of Glaucoma Syndromes

Genes have also been identified in various glaucoma syndromes in the Pakistani population. We conducted a study of 14 Pakistani families presented with Axenfeld Rieger Syndrome along with presentation of Glaucoma as one of the complications of the syndrome. A novel homozygous deletion (c.92_100del; p.Ala31_Ala33del) was identified in the *FOXC1* segregating in a family with congenital glaucoma presentation [49]. Another family carried a de novo mutation c.225C>A; p.Tyr75* in *PAX6* causative of glaucoma in syndromic form. The mutation was not present among the parents of the proband [49]. We also identified a novel heterozygous missense mutation c.2368T>A; p. Cys790Ser in *FBN1* in a Marfan's syndrome family of Pakistani origin [50].

The familial form of PACG is rare, though in our panel of glaucoma families, we identified two small families that were screened for plausible

genes, but no known or novel variants were identified in these families [Ayub et al. unpublished data], thus indicating involvement of novel genes in PACG families.

18.6.4 Genetics of Sporadic Glaucoma

In order to determine a comprehensive genetic overview of glaucoma in the Pakistani population, our group conducted a number of case-control association studies that resulted in identification of pathogenic as well as protective SNPs in the Pakistani population (Fig. 18.2). GWAS conducted for the identification of genetic risk factors of sporadic glaucoma subtype PACG in worldwide and Pakistani PACG patients resulted in the identification of five novel loci, *EPDR1* (rs3816415) *CHAT* (rs1258267), *GLIS3* (rs736893), *FERMT2* (rs7494379), and *DPM2–FAM102A* (rs3739821) to be causative of PACG globally, however, in the Pakistani population only *CHAT* (rs1258267) was significantly associated [31]. While another GWAS of sporadic PEXG [32], revealed novel loci; 13q12 (*POMP*), 11q23.3 (*TMEM136*), 6p21 (*AGPAT1*), 3p24 (*RBMS3*), and 5q23 (near *SEMA6A*) along with a rare protective allele at *LOXL1* (p.Phe407) [32]. Replication association studies revealed variants in *ASB10* to be associated with POAG [51], *TMCO1* (rs4656461) with POAG, PACG as well as PEXG, *ATOH7* (rs1900004) with PACG, and *CAV1* (rs4236601) with POAG [52]. Polymorphisms rs25487 in *XRCC1* and rs13181 in *XPD* were found to increase the risk of POAG in males [53], while *LOXL1* SNPs rs1048661 and rs3825942 did not show any gender bias and were found to be only associated with PEXG [54], while a 27-bp intron 4 VNTR polymorphism in *NOS3* and *HSP70* rs1043618 polymorphism were found to be associated with POAG and PACG [55], as well as with PEXG [Ayub et al. unpublished]. SNP rs3753841 in *COL11A1* was also found to be significantly associated with PACG [Ayub et al. unpublished data]. *TNFα* polymorphism G-308A was associated with PEXG [56], while rs74315341 and the novel SNP rs879255525 in *MYOC* increase the risk of POAG in the Pakistani population [33]. *MMP1* polymorphism rs1799750 was found associated with POAG, *MMP9* polymor-

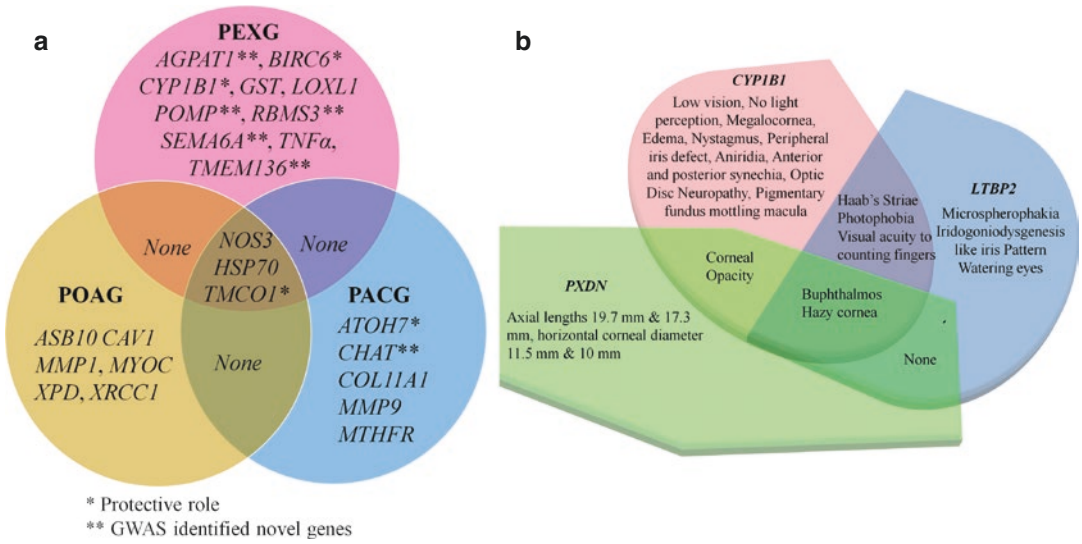

Fig. 18.2 (**a**) Genotype and phenotype correlations among the PCG families linked to the three genes *CYP1B1, PXDN, LTBP2* in the Pakistani population. (**b**) Genes, associated with three subtypes of Glaucoma: PEXG, POAG, and PACG, in the Pakistani Population

phism rs17576 with PACG [57]. *MTHFR* C677T polymorphism was found to be associated with PCAG but not POAG [58]. Whereas the *BIRC6* polymorphism rs2754511 was found to play a protective role in PEXG [59], and *CYP1B1* (*P450*) polymorphism rs2567206 also played a protective role in PEXG [Ayub et al. unpublished]. Moreover, *GSTT1* and *GSTM1* null genotypes were also found to be associated with PEXG in the Pakistani cohort [60].

There were few SNPs such as rs11720822 in *PDIA5* [59], rs11258194 in *OPTN, P21* polymorphism rs1801270, *P450* c.-2805T>C (POAG and PACG only), *CYP1B1* polymorphism rs2567206, rs1015213 in *PCMTD1,* rs11024102 polymorphism in *PLEKHA* that were not found to be associated with glaucoma in Pakistani population, despite their disease association in other ethnicities worldwide [Ayub et al. unpublished].

Polymorphic genetic variations in different genes that were observed to play a genetic role in sporadic glaucoma in the Pakistani population are listed in Table 18.2.

18.7 Pathology and Clinical Features

Different forms of glaucoma share some common clinical features that include changes in cup-to-disc ratio (CDR), thinning of retinal nerve fiber, which happens due to the loss of optic nerve and RGCs. The visual field loss initiates in the periphery until only the central vision is left [27]. Various forms of glaucoma exist in Pakistan, the most common clinical presentation among the children is PCG while POAG is common in adults. Based on the genetic findings there exists a genotype–phenotype correlation in different forms of glaucoma in Pakistan.

18.7.1 Genotype–Phenotype Correlation

Like genetic heterogeneity, the clinical presentation of glaucoma was also observed to be heterogeneous in the Pakistani glaucoma patients (Fig. 18.2b). In familial glaucoma subjects, Waryah et al. [42] observed varying degrees of onset and severity of disease in their studied families, where most of the families had early disease onset. In our cohort, a large family of POAG with a dominant mode of inheritance, had differences in the age of onset of the disease among various affected individuals among the sub-branches of the family. One sub-branch displayed early onset of the disease with an average age of 25 years while the other sub-branch had late disease onset with mean age of 50 years. The patients had severe phenotype with raised IOP, pain in eyes and forehead region and tunnel vision. The symptoms were severe in one of the patients with early onset of the disease; the affected girl had onset in the first decade of life with severe clinical symptoms and rapid loss of vision [Ayub et al. unpublished]. Another dominant late-onset POAG family had blurring of vision and persistent headache at the beginning of the disease. They had high intraocular pressure (IOP), retinal nerve fiber layer (RNFL) thinning, and appearance of clinical symptoms in fourth to fifth decade of life. However, a child (7 years at the time of sampling) in the family had loss of vision with excessively raised IOP and deep cupping, with appearance of clinical symptoms in the first decade of life. The child was also observed to be homozygous for the identified segregating mutation in *PHKG* while other affected individuals in this family were heterozygous [Ayub et al. unpublished data]. The families that were linked to *CYP1B1* had varying clinical phenotypes, one of them with a mutation (p.Arg390His) in *CYP1B1* had family members affected with PCG, JOAG, and late-onset POAG as well. "The patient having PCG had megalocornea, hazy cornea, edema, raised IOP (32 mmHg) and nystagmus, whereas the patients with late-onset POAG in the same family had poor vision, a phthisical left eye, nystagmus and optic disc neuropathy with a pigmentary mottling of the fundus in the macula of her right eye. The individual with JOAG onset had phthisical left eye, a peripheral iris defect, aniridia and anterior and posterior synechia, and a high IOP

(44 mmHg) of the right eye, while another individual had megalocornea, nystagmus, raised IOP in both eyes (35 and 30 mmHg) and a CDR of 1.0 in both eyes" [38]. In the same study, 4 PCG families were linked to *CYP1B1* (Fig. 18.2b). The age of onset was before 3 years with very high IOP (>40 mmHg), bulging eyes (buphthalmus), with varying opacity, horizontal corneal diameters were 13 mm [38] (Fig. 18.2b). Clinical variability has been observed among patients with variants in *CYP1B1* in the Pakistani population [41] (Fig. 18.2b). A PCG family with two affected individuals who suffered from glaucoma in first year of life had raised IOP (>40 mmHg) and nystagmus bilaterally. The family was genetically analyzed by homozygosity mapping that resulted in identification of a novel locus [Ayub et al. 2019 unpublished]. Both individuals had bulging avascularized and opaque corneas with sensitivity to touch and Haab's striae (horizontal breaks in the Descemet's membrane). The disease was progressive that resulted in complete blindness while the unaffected siblings had no signs of glaucoma. In other populations worldwide, patients with *PXDN* mutations have been observed to display severe anterior segment dysgenesis and microphthalmia [61], however, in our Pakistani cohort we observed anterior segment dysgenesis, sclerocornea, microphthalmia, hypotonia, and developmental delays among the patients [38]. Moreover, overlapping clinical features were observed among the probands of the PCG families that were linked to different genes (Fig. 18.1). Familial glaucoma clinical presentation is therefore observed to be complex where single gene defect results in differential phenotype among the family members, which points to the involvement of genetic modifiers in glaucoma progression, therefore, there is a need to identify these modifier genes to add to the understanding of the genetic etiology and hence the molecular mechanisms of glaucoma.

Among the sporadic cases that we studied, most of the patients came to the clinics with compromised vision. In the studied sporadic cohort, we observed comorbidity of NTG and high tension glaucoma (HTG) among the POAG group. Among these patients, the CDR ranged from 0.4 to 1 with increased vision loss along with increasing CDR. The patients with late-onset POAG were mostly blind due to glaucoma when they first visited the clinics. The JOAG patients usually had a family history and raised IOP with disturbed CDR (>0.5). Among the PACG patients, the IOP was observed to be very high, i.e., above 40 mmHg with red and watery eyes. The PACG patients usually had severe loss of vision, which in some cases was accompanied by excessive optic nerve damage, whereas patients of PEXG had exfoliation deposits in the TM and other aqueous bathed surfaces and usually had late-onset open angle glaucoma with raised IOP (<30) and disturbed CDR (>0.5).

The sporadic Pakistani glaucoma patient's awareness about glaucoma onset, progression and its consequences were poor as compared to the patients with family history. The former came to the clinics usually with compromised vision and poor understanding of the fact that vision restoration is not possible for a glaucoma patient and thus it becomes a major cause of depression among glaucoma patients.

Better understanding of the pathology of glaucoma therefore can help in improvement in management and treatment of the disease. In Pakistan, treatment generally includes medication, targeting, and reducing IOP by topical medicine, laser or surgical procedure [62]. β-blockers (timolol and betaxolol) are most commonly used to lower the IOP, along with α2 adrenoreceptors, which lower the IOP by inhibiting the aqueous humor inflow [63]. In case of failure of medicines and laser treatment or in very severe disease condition, trabeculectomy is the procedure of choice to lower the IOP and trabeculectomy with 5-FU is an efficient surgical procedure for glaucoma treatment in practice in Pakistan [64]. However, despite multiple treatment methods it has been observed that a number of patients respond to the medication differently, being categorized as responders and nonresponders to treatment. Such observations, therefore, point toward the need of exploring pharmacogenetic aspect of glaucoma in the Pakistani patients.

18.8 Molecular Biology

18.8.1 Molecular Biology of Familial Glaucoma in Pakistan

The major gene contributing to PCG in the Pakistani population and other ethnicities worldwide is observed to be *CYP1B1*. However, in the Pakistani population, we also observed *CYP1B1* mutations in POAG families thus extending the disease spectrum of *CYP1B1* to glaucoma subtypes. The gene belongs to the family of cytochrome *P450* [65] and the protein is essential in the proper development and functioning of the iridocorneal angle of the eye [66] and maintenance of the trabecular meshwork (TM), which is the most significant tissue with respect to glaucoma [67]. The mutated *CYP1B1* is predicted to result in disruption of TM cell arrangement at early developmental stages that interrupts the aqueous humor outflow resulting in elevated IOP in PCG as well as POAG patients [67]. The involvement of *CYP1B1* mutations in a POAG family that we studied where patients displayed clinical variability (late-onset POAG, JOAG, and PCG), points toward the involvement of a modifier gene, or it might be due to the interaction of environmental factors (xenobiotics or mutagenic chemicals) [68].

Mutations in *LTBP2* and *PXDN* (also reported in the Cambodian population [69]) have been found to be causative of PCG in the Pakistani population. LTBP2 is expressed in the TM ciliary processes where it has a vital function in the production and maintenance of aqueous humor, it is also involved in tissue repair and cell to cell adhesion [70]. The involvement of *LTBP2* in the developing elastic tissues is postulated as the molecular mechanism causative of PCG [71]. *LTBP2* also interacts with *FBN1* (which causes Marfan's syndrome) [72, 73] to maintain integrity of the extracellular matrix (ECM) [74]. Though the pathogenic mechanism for both LTBP2 and PXDN is unclear in glaucoma but they are both predicted to be linked to each other through COL4A2 and the assembly of ECM in the TM which is important in maintenance of IOP. *PRPF8*, is another gene, mutations in which have been found to be causative of POAG [46], this gene was previously reported as RP causing gene in retinal dystrophy families [75], this is a novel genetic association for POAG manifestation. *PRPF8* interacts at its C and N terminals with the interacting partners (U2-dependent spliceosome complex composed of four snRNPs; U1, U2, U4/U6, and U5), which are important for splicing. Previously C terminus mutations in the gene were identified to be causative of RP [75] but in our cohort we found mutations in the N terminus to be causative of POAG [46], thus mutations on both termini are predicted to disrupt the normal protein function and disturb the interaction with other proteins, however, how the N and C terminus mutations result in different phenotype needs further investigation.

Despite the fact that a number of genes have been identified in Pakistani glaucoma families, the unsolved families (51%; Fig. 18.1) indicate the existence of un-identified gene(s) and pathways causative of glaucoma.

18.8.2 Molecular Biology of Sporadic Glaucoma in Pakistan

The two major primary forms of glaucoma, PACG and POAG have been extensively studied worldwide as well as in the Pakistani population, however, we extended our sporadic glaucoma cohort to a secondary form of glaucoma, i.e., PEXG. We reported our first association study on *MTHFR* in 2008 [58], with PACG and not with POAG, which was also the first genetic association report of sporadic glaucoma phenotype from Pakistan. The difference in the association of *MTHFR* for different glaucoma subclasses led us to explore further, the other genetic variants involved in various molecular pathways that have reported association in other populations worldwide. We studied various SNPs in those genes that had shown association in ER stress and apoptosis (*P53*, *P21*, *P450*, *PDIA5*, *BIRC6*, *OPTN*), genes expressed in Ciliary body, TM (*MYOC*, *ASB10*), genes involved in overcom-

ing oxidative stress (*eNOS*, *HSP70*, *GSTs*). Cell junction maintenance (*PLEK1*), collagen growth and repair (*COLL11A1*, *MMPs*), DNA repair pathway genes (*XRCC*, *XPD*), and inflammation (*PCMTD1*, *TNFα*).

These association studies helped us in the identification of population-specific SNPs. A global screening of PACG samples identified novel genes and pathways [31] in which five novel loci were found to be associated with PACG (Table 18.2). When population-based data were analyzed, only one SNP (*CHAT*; rs1258267) was found to be associated with PACG in the Pakistani population [31]. Moreover, when the global PEXG cohorts were screened, it also resulted in novel loci identification in the Pakistani population as well [32]. Thus, these studies helped in highlighting the pathways such as oxidative stress and inflammatory pathway that are associated with the disease in the Pakistani population. Further replication studies should be done in Pakistani as well as in other populations for the identification of population-specific genetic risk variants.

18.9 Summary

Pakistan, with one of the highest ratios of consanguinity, has a large number of genetic disease families including glaucoma. There is clinical as well as genetic variability among the patients of glaucoma in the Pakistani population. In certain cases, a single gene mutation might cause varying effects in the individuals, suggestive of the presence of modifiers that require further comprehensive studies to understand the complete mechanisms of glaucoma. Moreover, glaucoma awareness is the need of the hour and all modes of communication should be used to sensitize the public at large. The patients are not familiar with the genetic basis and the familial nature of the disease; therefore it has become a serious blindness-related issue in Pakistan despite advances in diagnostic, medical, and surgical treatment options.

Moreover, looking at the mutation spectrum of *CYP1B1* and the identification of novel mutations in the gene in Pakistani population, this gene should be prescreened for mutations in all new families of not only PCG but also POAG. Several novel mutations in novel and known genes identified in our cohort could not be functionally validated in the genetic studies conducted in Pakistan. Therefore, molecular characterization of glaucoma is essential, which can only be done through high throughput techniques. As glaucoma is a complex disorder therefore the best technique for its molecular characterization is whole genome sequencing, which will be helpful in understanding its genetic etiology. Functional studies including proteomic approaches and animal models would further be required for the validation of the genetic data, followed by further replication studies in different populations. Only then it would be possible to unravel the causative agents of these complex disorders and their underlying molecular mechanisms that will further help in developing better therapeutic interventions.

References

1. Faucher M, Anctil JL, Rodrigue MA, Duchesne A, Bergeron D, Blondeau P, Cote G, Dubois S, Bergeron J, Arseneault R, Morissette J, Raymond V. Founder TIGR/myocilin mutations for glaucoma in the Quebec population. Hum Mol Genet. 2002;11:2077–90.
2. Baird PN, Craig JE, Richardson AJ, Ring MA, Sim P, Stanwix S, Foote SJ, Mackey DA. Analysis of 15 primary open-angle glaucoma families from Australia identifies a founder effect for the Q368STOP mutation of myocilin. Hum Genet. 2003;112:110–6.
3. Pang CP, Leung YF, Fan B, Baum L, Tong WC, Lee WS, Chua JK, Fan DS, Liu Y, Lam DS. TIGR/MYOC gene sequence alterations in individuals with and without primary open-angle glaucoma. Invest Ophthalmol Vis Sci. 2002;43:3231–5.
4. Lander ES, Schork NJ. Genetic dissection of complex traits. Science. 1994;265:2037–48.
5. Alward WL, Fingert JH, Coote MA, Johnson AT, Lerner SF, Junqua D, Durcan FJ, Mccartney PJ, Mackey DA, Sheffield VC, Stone EM. Clinical features associated with mutations in the chromosome 1 open-angle glaucoma gene (GLC1A). N Engl J Med. 1998;338:1022–7.
6. Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol. 2006;90:262–7.
7. Tham YC, Li X, Wong TY, Quigley HA, Aung T, Cheng CY. Global prevalence of glaucoma and pro-

jections of glaucoma burden through 2040: a systematic review and meta-analysis. Ophthalmology. 2014;121:2081–90.
8. Cook C, Foster P. Epidemiology of glaucoma: what's new? Can J Ophthalmol. 2012;47:223–6.
9. Fuse N. Genetic bases for glaucoma. Tohoku J Exp Med. 2010;221:1–10.
10. Liu Y, Allingham RR. Molecular genetics in glaucoma. Exp Eye Res. 2011;93:331–9.
11. Sarfarazi M, Stoilov I, Schenkman JB. Genetics and biochemistry of primary congenital glaucoma. Ophthalmol Clin North Am. 2003;16:543–54.
12. Bejjani BA, Stockton DW, Lewis RA, Tomey KF, Dueker DK, Jabak M, Astle WF, Lupski JR. Multiple CYP1B1 mutations and incomplete penetrance in an inbred population segregating primary congenital glaucoma suggest frequent de novo events and a dominant modifier locus. Hum Mol Genet. 2000;9:367–74.
13. Mozaffarieh M, Grieshaber MC, Flammer J. Oxygen and blood flow: players in the pathogenesis of glaucoma. Mol Vis. 2008;14:224–33.
14. Coleman AL. Glaucoma. Lancet. 1999;354:1803–10.
15. Vithana EN, Khor CC, Qiao C, Nongpiur ME, George R, Aung T, et al. Genome-wide association analyses identify three new susceptibility loci for primary angle closure glaucoma. Nat Genet. 2012;44:1142–6.
16. Shastry BS. Genetic susceptibility to primary angle closure glaucoma (PACG). Discov Med. 2013;15:17–22.
17. Challa P. Genetics of adult glaucoma. Int Ophthalmol Clin. 2011;51:37–51.
18. Memon MS. Prevalence and causes of blindness in Pakistan. J Pak Med Assoc. 1992;42(8):196–8.
19. Dineen B, Bourne RR, Jadoon Z, Shah SP, Khan MA, Foster A, Gilbert CE, Khan MD. Pakistan National Eye Survey Study Group. Causes of blindness and visual impairment in Pakistan. The Pakistan national blindness and visual impairment survey. Br J Ophthalmol. 2007;91:1005–10.
20. Iqbal S, Khan Z, Shah AS, Khan MY. Types and presentation of glaucoma. JPMI. 2008;22:324–32.
21. Akhtar F, Micheal S, Khan MI, Yousaf S, Bilal M, Ahmed A, Qamar R. Does gender have an effect in the Prevalence of types of glaucoma in Pakistani population? ASJO. 2010;6:30–6.
22. Lewis CJ, Hedberg-Buenz A, DeLuca AP, Stone EM, Alward W, Fingert JH. Primary congenital and developmental glaucomas. Hum Mol Genet. 2017;26:28–36.
23. Bener A, Mohammad AR. Global distribution of consanguinity and their impact on complex diseases: Genetic disorders from an endogamous population. Egypt J Med Hum Genet. 2017;18:315–20.
24. Mitchell P, Smith W, Atteno K, Healy P. Prevalence of open-angle glaucoma in Australia: The Blue Mountains Eye Study. Ophthalmology. 1996;103:1661–9.
25. Cascella R, Strafella C, Germani C, Novelli G, Ricci F, Zampatti S, Giardina E. The genetics and the genomics of primary congenital glaucoma. BioMed Res Int. 2015;3:212–91.
26. Jabeen S, Mahsood Y, Sadiq M, Akhtar F. Causes clinical behavior and final outcome of steroid induced glaucoma. ASJO. 2006;12:110–4.
27. Fasih U, Fehmi MS, Shaikh N, Shaikh A. Secondary glaucoma-causes and management. Pak J Ophthalmol. 2008;24:86–92.
28. Shahid M, Iram M, Rafique F, Mehwish HH, Shehzadi N, Hayat S. Awareness and knowledge about glaucoma among rural population of Punjab. Pak J Med Health Sci. 2017;11:1124–6.
29. Wiggs JL. Genetic etiologies of glaucoma. Arch Ophthalmol. 2007;125:30–7.
30. Ismail AHM, Chaudhry S, Akhtar F. Compliance to medical treatment in patients of chronic simple glaucoma: study of factors causing non-compliance. IMJ. 2015;07:77–81.
31. Khor CC, Do T, Jia H, Nakano M, George R, Abu-Amero K, Duvesh R, Chen LJ, Li Z, Nongpiur ZE, et al. Genome-wide association study identifies five new susceptibility loci for primary angle closure glaucoma. Nat Genet. 2016;48:556–62.
32. Aung T, Ozaki M, Lee MC, Schlötzer-Schrehardt U, Thorleifsson G, Mizoguchi T, et al. Genetic association study of exfoliation syndrome identifies a protective rare variant at LOXL1 and five new susceptibility loci. Nat Genet. 2017;49:993–1004.
33. Nazir S, Mukhtar M, Shahnawaz M, Farooqi S, Fatima N, Mehmood R, Sheikh N. A novel single nucleotide polymorphism in exon 3 of MYOC enhances the risk of glaucoma. PLoS One. 2018;13:e0195157.
34. Bashir R, Sanai M, Azeem A, Altaf I, Saleem F, Naz S. Contribution of GLC3A locus to primary congenital glaucoma in Pakistani population. Pak J Med Sci. 2014;30:1341–5.
35. Rauf B, Irum B, Kabir F, Firasat S, Naeem MA, Khan SN, Husnain T, Riazuddin S, Akram J, Riazuddin SA. A spectrum of CYP1B1 mutations associated with primary congenital glaucoma in families of Pakistani descent. Hum Genome Var. 2016;3:16–21.
36. Chouiter L, Nadifi S. Analysis of *CYP1B1* gene mutations in patients with primary congenital glaucoma. J Pediatr Genet. 2017;6:205–14.
37. Firasat S, Riazuddin SA, Khan SN, Riazuddin S. Novel CYP1B1 mutations in consanguineous Pakistani families with primary congenital glaucoma. Mol Vis. 2008;14:2002–9.
38. Micheal S, Ayub H, Zafar SN, Bakker B, Ali M, Akhtar F, Islam F, Khan MI, Qamar R, den Hollander AI. Identification of novel CYP1B1 gene mutations in patients with primary congenital and primary open-angle glaucoma. Clin Experiment Ophthalmol. 2015;43:31–9.
39. Sheikh SA, Waryah AM, Narsani AK, Shaikh H, Gilal IA, Shah K, Qasim M, Memon AI, Kewalramani P, Shaikh N. Mutational spectrum of the CYP1B1 gene in Pakistani patients with primary congenital glaucoma: novel variants and genotype-phenotype correlations. Mol Vis. 2014;20:991–1001.
40. Afzal R, Firasat S, Kaul H, Ahmed B, Siddiqui SN, Zafar SN, Shahzadi M, Afshan K. 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. Congenit Anom (Kyoto). 2018; https://doi.org/10.1111/cga.12312.
41. Bashir R, Yousaf K, Tahir H, Sanai M, Qayyum S, Naz S, Naz S. Clinical variability of CYP1B1 gene variants in Pakistani primary congenital glaucoma families. J Pak Med Assoc. 2018;68:1205–11.
42. Waryah YM, Iqbal M, Sheikh SA, Baig MA, Narsani AK, Atif M, Bhinder MA, Rahman A, Memon AI, Pirzado MS, Waryah AM. Two novel variants in CYP1B1 gene: a major contributor of autosomal recessive primary congenital glaucoma with allelic heterogeneity in Pakistani patients. Int J Ophthalmol. 2019;12:8–15.
43. Bashir R, Tahira H, Yousaf K, Naz S, Naz S. Homozygous p.G61E mutation in a consanguineous Pakistani family with co-existence of juvenile-onset open angle glaucoma and primary congenital glaucoma. Gene. 2015;570:295–8.
44. Ali M, Mckibbin M, Booth A, Parry DA, Jain P, Riazuddin SA, Hejtmancik JF, Khan SN, Firasat S, Shires M, Gilmour DF, Towns K, Murphy AL, Azmanov D, Tournev I, Cherninkova S, Jafri H, Raashid Y, Toomes C, Craig J, Mackey DA, Kalaydjieva L, Riazuddin S, Inglehearn CF. Null mutations in LTBP2 cause primary congenital glaucoma. Am J Hum Genet. 2009;84:664–71.
45. Micheal S, Siddiqui SN, Zafar SN, Iqbal A, Khan MI, den Hollander AI. Identification of novel variants in LTBP2 and PXDN using whole-exome sequencing in developmental and congenital glaucoma. PLoS One. 2016;11:e0159259.
46. Micheal S, Hogewind BF, Khan MI, Siddiqui SN, Zafar SN, Akhtar F, den Hollander AI. Variants in the PRPF8 gene are associated with glaucoma. Mol Neurobiol. 2018;55:4504–10.
47. Waryah AM, Narsani AK, Sheikh SA, Shaikh H, Shahani MY. The novel heterozygous Thr377Arg MYOC mutation causes severe Juvenile Open Angle Glaucoma in a large Pakistani family. Gene. 2013;528:356–9.
48. Firasat S, Riazuddin SA, Hejtmancik JF, Riazuddin S. Primary congenital glaucoma localizes to chromosome 14q24.2-24.3 in two consanguineous Pakistani families. Mol Vis. 2008;14:1659–65.
49. Micheal S, Siddiqui SN, Zafar SN, Villanueva-Mendoza C, Cortés-González V, Khan MI, den Hollander AI. A novel homozygous mutation in FOXC1 causes Axenfeld Rieger Syndrome with congenital glaucoma. PLoS One. 2016;11:e0160016.
50. Micheal S, Khan MI, Akhtar F, Weiss MM, Islam F, Ali M, Qamar R, den Hollander AI. Identification of a novel FBN1 gene mutation in a large Pakistani family with Marfan syndrome. Mol Vis. 2012;18:1918–26.
51. Micheal S, Ayub H, Islam F, Siddiqui SN, Khan WA, Akhtar F, Qamar R, Khan MI, den Hollander AI. Variants in the ASB10 gene are associated with primary open angle glaucoma. PLoS One. 2015;10:e0145005.
52. Micheal S, Ayub H, Khan MI, Bakker B, Schoenmaker-Koller FE, Ali M, den Hollander AI. Association of known common genetic variants with primary open angle primary angle closure and pseudoexfoliation glaucoma in Pakistani cohorts. Mol Vis. 2014;20:1471–9.
53. Yousaf S, Khan MI, Micheal S, Akhtar F, Ali SHB, Riaz M, Ahmed A. XRCC1 and XPD DNA repair gene polymorphisms: a potential risk factor for glaucoma in the Pakistani population. Mol Vis. 2011;17:1153–63.
54. Micheal S, Khan MI, Akhtar F, Ali M, Ahmed A, den Hollander AI, Qamar R. Role of Lysyl oxidase-like 1 gene polymorphisms in Pakistani patients with pseudoexfoliative glaucoma. Mol Vis. 2012;18:1040–4.
55. Ayub H, Khan MI, Micheal S, Akhtar F, Ajmal M, Shafique S, Qamar R. Association of eNOS and HSP70 gene polymorphisms with glaucoma in Pakistani cohorts. Mol Vis. 2010;16:18–25.
56. Khan MI, Micheal S, Rana N, Akhtar F, den Hollander AI, Ahmed A, Qamar R. Association of tumor necrosis factor alpha gene polymorphism G-308A with pseudoexfoliative glaucoma in the Pakistani population. Mol Vis. 2009;15:2861–7.
57. Micheal S, Yousaf S, Khan MI, Akhtar F, Islam F, Khan WA, den Hollander AI, Qamar R, Ahmed A. Polymorphisms in matrix metalloproteinases MMP1 and MMP9 are associated with primary open-angle and angle closure glaucoma in a Pakistani population. Mol Vis. 2013;19:441–7.
58. Michael S, Qamar R, Akhtar F, Khan WA, Ahmed A. C677T polymorphism in the methylenetetrahydrofolate reductase gene is associated with primary closed angle glaucoma. Mol Vis. 2008;14:661–5.
59. Ayub H, Micheal S, Akhtar F, Khan MI, Bashir S, Waheed NK, Ali M, Schoenmaker-Koller FE, Shafique S, Qamar R, den Hollander AI. Association of a polymorphism in the BIRC6 gene with pseudoexfoliative glaucoma. PLoS One. 2014;9:e105023.
60. Khan MI, Micheal S, Akhtar F, Ahmed W, Ijaz B, Ahmed A, Qamar R. The association of glutathione S-transferase GSTT1 and GSTM1 gene polymorphism with pseudoexfoliative glaucoma in a Pakistani population. Mol Vis. 2010;16:2146–52.
61. Choi A, Lao R, Ling-Fung TP, Wan E, Mayer W, Bardakjian T, Shaw GM, Kwok PY, Schneider A, Slavotinek A. Novel mutations in PXDN cause microphthalmia and anterior segment dysgenesis. EJHG. 2014;23:337–41.
62. Khan L, Ali M, Qasim M, Jabeen F, Hussain B. Molecular basis of glaucoma and its therapeutical analysis in Pakistan: an overview. Biomed Res Ther. 2017;4:1210–27.
63. Brooks AM, Gillies WE. Ocular beta-blockers in glaucoma management. Clinical pharmacological aspects. Drugs Aging. 1992;2:208–21.
64. Yousaf M, Hussain A, Muhammad N, Irfan U, Farooq S, Sadiq M, Akhtar F. Trabeculectomy versus combined trabeculectomy and cataract surgery in pseudoexfoliation glaucoma. ASJO. 2016;12:131–4.

65. Zhao Y, Wang S, Sorenson CM, Teixeira L, Dubielzig RR, Peters DM, Conway SJ, Jefcoate CR, Sheibani N. CYP1B1 mediates periostin regulation of trabecular meshwork development by suppression of oxidative stress. Mol Cell Biol. 2013;33:4225–40.
66. Sarfarazi M. Recent advances in molecular genetics of glaucomas. Hum Mol Genet. 1997;6:1667–77.
67. Faiq MA, Dada R, Qadri R, Dada T. CYP1B1-mediated pathobiology of primary congenital glaucoma. J Curr Glaucoma Pract. 2016;9:77–80.
68. Melki R, Colomb E, Lefort N, Brézin AP, Garchon HJ. CYP1B1 mutations in French patients with early-onset primary open-angle glaucoma. J Med Genet. 2004;41:647–51.
69. Khan K, Rudkin A, Parry DA, Burdon KP, McKibbin M, Logan CV, Abdelhamed ZI, et al. Homozygous mutations in PXDN cause congenital cataract corneal opacity and developmental glaucoma. Am J Med Sci. 2011;89:464–73.
70. Shipley JM, Mecham RP, Maus E, Bonadio J, Rosenbloom J, McCarthy RT, et al. Developmental expression of latent transforming growth factor β binding protein 2 and its requirement early in mouse development. Mol Cell Biol. 2000;20:4879–87.
71. Gibson MA, Hatzinikolas G, Davis EC, Baker E, Sutherland GR, Mecham RP. Bovine latent transforming growth factor beta 1-binding protein 2: molecular cloning identification of tissue isoforms and immunolocalization to elastin-associated microfibrils. Mol Cell Biol. 1995;15:6932–42.
72. Hirai M, Horiguchi M, Ohbayashi T, Kita T, Chien KR, Nakamura T. Latent TGF-beta-binding protein 2 binds to DANCE/fibulin-5 and regulates elastic fiber assembly. EMBO J. 2007;26:3283–95.
73. Désir J, Sznajer Y, Depasse F, et al. LTBP2 null mutations in an autosomal recessive ocular syndrome with megalocornea spherophakia and secondary glaucoma. Eur J Hum Genet. 2010;18:761–7.
74. Vehvilainen P, Hyytiainen M, Keski-Oja J. Matrix association of latent TGF-beta binding protein-2 (LTBP-2) is dependent on fibrillin-1. J. Cell. Physiol. 2009;221:586–93.
75. McKie AB, McHale JC, Keen TJ, Tarttelin EE, Goliath R, Lith-Verhoeven JJ, Greenberg J, Ramesar RS, et al. Mutations in the pre-mRNA splicing factor gene PRPC8 in autosomal dominant retinitis pigmentosa (RP13). Hum Mol Genet. 2011;10:1555–62.