Primary Congenital Glaucoma Genetics: The Experience in Brazil

17

Mônica Barbosa de Melo,
José Paulo Cabral de Vasconcellos,
and Vital Paulino Costa

Abstract

Primary congenital glaucoma (PCG) is the most prevalent form among childhood glaucomas, with an incidence varying between 1:1250 and 1:30,000. The majority of PCG cases are sporadic and families have been reported with an autosomal recessive inheritance pattern and variable penetrance. Genetic heterogeneity has been observed in PCG. Five loci have been identified (*GLC3A–GLC3E*) and, among these loci, variants in three genes have been associated with PCG.

In Brazil, to date the genetic profile of PCG is restricted to the evaluation of the *CYP1B1* gene. Brazil is a country characterized by a highly admixed population and low frequency of consanguineous marriages. Most studies have been conducted in the Southeast and report a frequency of disease-associated variants ranging from 23.5 to 50.0%, with most variants present in compound heterozygosity and some variants still unique to this population. An association between variants in the *CYP1B1* gene and poor prognosis has also been observed, reinforcing the importance of investigating this gene in Brazilian PCG patients.

Keywords

Primary congenital glaucoma · Genetics
CYP1B1 · Gene · Brazil

17.1 Introduction

Glaucoma comprises several conditions that affect the optic nerve leading to structural changes characterized by loss of retinal nerve fiber layer and optic disc cupping with corresponding visual field defects. The pathophysiology of glaucoma involves many combined mechanisms and metabolic pathways, but the main one includes the increase of intraocular pressure (IOP). IOP is determined by the equilibrium of aqueous humor production at the ciliary body and its outflow through the trabecular meshwork (conventional outflow) and ciliary muscle fibers (uveoscleral outflow). In general terms, the glaucomas are classified according to their etiology, anterior chamber anatomy, and age of onset. In respect to etiology, glaucoma can be divided into primary (with no identified cause) and secondary to an

M. Barbosa de Melo (✉)
Center for Molecular Biology and Genetic Engineering, University of Campinas, Campinas, São Paulo, Brazil

J. P. Cabral de Vasconcellos · V. Paulino Costa
Department of Ophthalmology and Otorhinolaryngology, School of Medical Sciences, University of Campinas, Campinas, São Paulo, Brazil

G. Prakash, T. Iwata (eds.), *Advances in Vision Research, Volume III*, Essentials in Ophthalmology,
https://doi.org/10.1007/978-981-15-9184-6_17

ocular or systemic condition. Glaucomas can also be classified according to the anatomic characteristic of the anterior chamber angle (open angle or closed angle), and finally depending on the age of onset (adult or pediatric) [1–3].

In 2013, an international consortium of glaucoma specialists named Childhood Glaucoma Research Network proposed a classification of pediatric glaucomas based on clinical aspects and in the context of clinical and ocular features in which the diagnosis was made. Therefore, both primary congenital glaucoma (PCG) and juvenile open-angle glaucoma are classified as primary childhood glaucomas, since none is associated with acquired ocular anomalies, systemic disease, or syndromes [4]. Among the pediatric glaucomas, PCG is the most prevalent, with an incidence varying between 1:1250 and 1:30,000, depending on the population that is investigated. In general, the more inbred the population, the higher the incidence [5].

17.2 Epidemiology

In Brazil, there is no robust study indicating the prevalence/incidence of PCG. Available data comes from studies performed at University Hospitals. One of these studies evaluated 72 children with pediatric glaucoma, showing 61.5% with PCG and 38.5% with secondary congenital glaucoma [6].

Other studies have evaluated the causes of visual impairment in children at low vision services in Brazilian public hospitals. It is important to emphasize that Brazil is a country with a continental dimension, with the North region being less developed than the South region. These socioeconomic characteristics lead to different frequencies in causes of visual impairment in children: infections tend to be the leading cause in the North, whereas retinopathy of prematurity is more frequent in the South. In a study that evaluated children from the rural area, the main causes of low vision were congenital cataract and toxoplasmosis (14.0%) followed by congenital glaucoma [7]. Another study, performed at the University of São Paulo, the most developed state in Brazil, involving 3210 cases, also showed macular toxoplasmosis as the first cause of visual impairment in children with low vision (20.7%), followed by retinal dystrophies (12.2%), retinopathy of prematurity (11.8%), ocular malformations (11.6%), and congenital glaucoma (10.8%) [8].

A recent update about the causes of childhood blindness worldwide situates glaucoma as the third leading cause in the Americas. Interestingly, glaucoma does not appear as one of the three main causes of blindness in the other regions of the world (Africa, Eastern Mediterranean, Europe, Southeast Asia, and Western Pacific) [9].

17.3 Mechanisms and Clinical Features

The presumed mechanism in PCG development is related to a dysfunction in the outflow system, particularly at the conventional outflow. Trabecular meshwork and Schlemm canal are both structures derived from the neural crest and mesodermal lineage. During the embryonic period, these tissues do not complete their maturation, which results in decreased outflow and IOP rise. The effect of increased IOP in ocular structures go beyond glaucomatous optic nerve damage, including ocular globe enlargement (buphthalmos), increase in corneal diameter (megalocornea), and breaks in the corneal endothelium (Haab striae). These ocular alterations lead to a classical clinical triad of GCP symptoms including epiphora, blepharospasm, and photophobia [10, 11].

The Childhood Glaucoma Research Network has introduced a classification guide to PCG diagnosis defined as the presence of two or more of the following criteria: IOP greater than 21 mmHg, optic disc cupping, corneal abnormalities related to PCG, ocular enlargement evaluated by axial length or progressive myopia, and visual field defect consistent with glaucoma [4]. The treatment of PCG aims at reducing IOP and controlling amblyopia. In the vast majority

of cases, IOP control requires initially an angular surgical procedure in order to improve aqueous humor outflow. PCG is mostly bilateral and asymmetrical with no sex preference in familial cases, but with higher male prevalence in sporadic cases [3, 10].

17.4 Genetic Aspects

The majority of PCG cases are sporadic and families have been reported with an autosomal recessive inheritance pattern and variable penetrance. Since the observation of inheritance patterns and the advances of molecular biology tools, several families have been evaluated, leading to the identification of genes associated with PCG. Genetic heterogeneity has been observed in PCG, what means that the same clinical phenotype results from variants in different loci/genes or that different patients, with the same genetic disease, present with different alterations in the same gene [3, 11]. Five loci have been identified (*GLC3A*–*GLC3E*) and, among these loci, variants in three genes have been associated with PCG [12].

Sarfarazi et al. identified the first gene, cytochrome P4501B1 (*CYP1B1*), located on chromosome 2p21 linked to *GLC3A* locus in Pakistani families [13, 14]. *CYP1B1* belongs to the cytochrome P450 family of membrane-bound oxidase enzymes and codes for P4501B1, a monooxygenase probably involved in the metabolism of a variety of substrates, including steroids and retinoids. Different from other P450 proteins, CYP1B1 is highly expressed outside the liver, particularly in tissues responsible for IOP homeostasis: trabecular meshwork and ciliary body [15, 16].

The mechanism through which disease-associated variants cause PCG is not completely understood. It is suggested that the enzyme codified by the gene would participate in metabolic pathways involved in the development of the anterior chamber, particularly, in the formation of the trabecular meshwork, via degradation of certain metabolites, as well as in the clearance of reactive oxygen species. Hence, variants in the *CYP1B1* gene could compromise the development and differentiation of this tissue, leading to IOP elevation and consequent optic nerve damage [17, 18].

The *CYP1B1* gene consists of three exons, one non-coding, and two introns [19]. More than 150 variants associated with PCG have been described, according to the "The Human Gene Mutation Database" (HGMD) [20]. The distribution of mutations can vary worldwide, from 14 to 30% in North American and European populations, from 15 to 20% in Chinese and Japanese populations and from 90 to 100% in Saudi Arabians and Slovakian Gypsies [3, 21–26]. The type of disease-associated variants can also be more frequent in certain populations. For example, E387K seems to be a founder variant in Slovakian Gypsies, G61E is a founder mutation in the Middle Eastern population and R390H is common among Asian populations [27].

Following the identification of *GLC3A* locus and its corresponding *CYP1B1* gene, two other loci, *GLC3B* and *GLC3C*, located on 1p36 and 14q24, respectively, were reported, but no PCG-associated variants have been identified [28, 29].

The *GLC3D* locus is also located on 14q24 and encompasses the latent transforming growth factor beta-binding protein 2 (*LTBP2* gene). *LTBP2* gene was identified through linkage analysis in Pakistani and Iranian PCG families presenting with autosomal recessive inheritance pattern. This gene encodes an extracellular matrix protein expressed in tissues with high concentration of elastic fibers with putative function in elastin microfibril assembly and cell adhesion. Its expression in ocular tissues such as the trabecular meshwork and ciliary body, as well as its role in anterior chamber development, make disease-causing variants in *LTBP2* gene a reasonable cause of PCG. Unlike the worldwide distribution of *CYP1B1* gene variants, *LTBP2* alterations have been reported in few populational groups [11, 30].

The most recently identified locus is *GLC3E*, which contains the tunica interna endothelial

cell kinase (*TEK*) gene. This gene was not found in a family-based linkage study, but in transgenic mice that harbored deletions in *TEK* gene or in both major angiopoietin ligands. These transgenic mice had a developmental loss of Schlemm's canal, resulting in IOP rise and ganglion cell loss compatible with a PCG phenotype. These findings led to a candidate gene approach involving 189 unrelated PCG patients, of whom ten presented heterozygous disease-causing variants in the human *TEK* gene [31].

17.4.1 *CYP1B1* Gene Screening in Brazilian PCG Patients

The first study that described the analysis of the *CYP1B1* gene in PCG Brazilian patients was a collaboration between two Brazilian Universities from the state of São Paulo and the group directed by Dr. Mansoor Sarfarazi [32]. Fifty-two patients were evaluated through single-strand conformation polymorphism and Sanger sequencing: 51.9% presented positive family history, consanguinity was reported by 26.9 and 84.6% had bilateral PCG. Fifty percent of the patients showed disease-associated variants. The majority of them were present in familial versus sporadic cases (55.6 versus 41.7%) and in bilateral versus unilateral disease (55.8 versus 12.5%). Homozygosity was reported in 57.7% of the cases, heterozygosity in 15.4%, and compound heterozygosity in 26.9%. Eleven different mutations have been identified, four of them described for the first time (g.3860C>T, g.4340delG, g.8165C>G and g.8214_8215delAG). The 4340delG variant was present in 46.0% (12/26) of PCG cases positive for *CYP1B1* alterations (nine homozygotes, two compound heterozygotes, and one heterozygote), associated with a severe phenotype, coursing with early onset (91.7% of the cases in the first month), worse clinical prognosis (all bilateral cases, high IOP in 11/12 cases), and limited response to surgical treatment. The most frequent haplotype observed among Brazilian patients was 5′-CCGGTA-3′, which was associated with at least seven mutations and probably with 4340delG.

In a report from Hollander et al., a deeper genotype–phenotype correlation for *CYP1B1* variants was performed. The trabeculectomy specimens from patients harboring variants were analyzed showing different extent of goniodysgenesis dependent on the genotype. One of the patients was a compound heterozygote for 4340delG and C209R. This patient showed severe goniodysgenesis, with agenesis of the Schelemm's canal [33].

In a joint study of Brazilian and American families, three variants in the *CYP1B1* gene have been identified in two Brazilian families (g.8037_8046dupTCATGCCACC in homozygosity, g.8182delG, and p.Glu387Lys in compound heterozygosity) by Sanger sequencing. In both pedigrees, the disease presented with corneal edema, early onset and high IOP. The same variants were reported in one of the American families (g.8037_8046dupTCATGCCACC and p.Glu387Lys), who also showed the p.268delSNF variant. Patients who harbor these variants shared a common haplotype, indicating a common founder between these two populations [34].

Della Paolera et al. conducted another study with 30 patients from the state of São Paulo [35]. PCG was bilateral in 66.7% of the cases and unilateral in 33.3%. All patients underwent a surgical procedure before the age of 3 months and all cases were sporadic, with no consanguineous marriage being reported. Thirty percent of the patients (9/30) presented *CYP1B1* variants, detected by Sanger sequencing and ten different mutations were described, two of them for the first time (4523delC and L378Q). Four of the patients presented variants in compound heterozygosis, two in homozygosis and in three patients only one mutant allele was identified. Prognosis was worse in patients who harbored alterations in the *CYP1B1* gene: mean IOP at diagnosis was higher, more surgical procedures were necessary for IOP control (the risk of patients positive for *CYP1B1* alterations to undergo more than one surgical procedure was nine times greater than the negative ones), and all patients had bilateral glaucoma.

Different from the first study conducted in the population from São Paulo state, the 4340delG variant was present in only two (6.7%) patients in heterozygosis. Two patients presented one of the new variants, 4635delT, in homozygosis. Both patients had severe bilateral disease, with two to three surgeries in each eye to control IOP and important visual function impairment. The 4523delC and L378Q alterations were present in compound heterozygosity in three members from the same family, all with high IOP at diagnosis, difficult surgical control and poor visual function.

Few years ago, a study involving Indian and Brazilian GCP patients evaluated 301 and 150 patients, respectively [36]. This study encompassed Brazilian patients from two previous studies as well as 68 new cases [32, 35]. A frequency of approximately 44.0% disease-associated variants in the *CYP1B1* gene has been reported in both populations. Despite the similar frequency, variants in homozygosis were more frequent in the Indian cohort (24.2% versus 16.7%) while compound heterozygosis was more frequent in the Brazilian cohort (12.7% versus 6.0%), which is probably due to the higher rate of consanguineous marriages among Indians. Both populations exhibited significant allelic heterogeneity. Thirty-nine variants were reported in Indian patients, while 17 in Brazilian patients. Most of these variants were population specific. Thirty-three were present only in Indian patients, while 11 were reported only in Brazilian patients. Six variants were shared between both groups (g.8037_8046dup10, g.8214_8215delAG, p.R368H, p.P437L, p.A443G, and p.S476P). The most prevalent alterations were R368H in India and 4340delG in Brazil. The R368H was observed in only three Brazilian patients (in homozygosity and compound heterozygosity) and the 4340delG was observed only in the Brazilian cohort. Regarding haplotype distribution, as observed in previous studies, the 5′-CCGGTA-3′ was a risk haplotype, associated with most variants.

In the group of Brazilian patients, 44.0% of the patients showed *CYP1B1* disease-associated variants. Although not statistically significant, age of onset was lower in the group positive for *CYP1B1* alterations. This group also showed higher frequency of family history and consanguinity. When all Brazilian PCG samples were evaluated (52 from the first study, 30 from the second study, and 68 from this study) no association was observed between alterations in the *CYP1B1* gene, IOP, and corneal diameter. In this report, the number of surgeries and number of affected eyes were not evaluated in relation to *CYP1B1* changes.

Another example of a patient from the Southeast Brazil is a 2-month-old male infant with bilateral PCG who was screened for variants in the *CYP1B1* gene. Glaucoma was diagnosed when he was less than 1 month old. The patient presented IOP of 26 mmHg in the right eye and 28 mmHg in the left eye, axial length of 21.49 mm in the right eye and 22.20 mm in the left eye, as well as buphthalmos, megalocornea, and corneal edema. The child has been submitted to four surgeries to control IOP. The *CYP1B1* gene screening showed the presence of two different variants (compound heterozygosity): p.E387K inherited from the father and p.R444Ter, inherited from the mother. As far as we know the R444Ter variant is being described for the first time in Brazil. The parents had no glaucoma or family history of glaucoma and no consanguinity (Fig. 17.1, data not published).

Recently, a study was conducted by Coêlho and collaborators who evaluated 17 PCG patients from an ethnically diverse population from the Northeast Brazil through next-generation sequencing [37]. Most of the patients had bilateral glaucoma (88.2%), the age at diagnosis ranged from 0 to 9 years and in 52.9% of the patients at least two surgical procedures were required. The late diagnosis reflects the poor health care quality in the Northeast compared to the Southeast region of Brazil. Disease-associated variants were present in 23.5% of the patients, three compound heterozygotes and one homozygote, and five different variants were reported, two of which were described for the first time in Brazilian patients (p.G61E and p.Y81N). No genotype–phenotype correlation was observed.

All variants that have been reported in Brazilian PCG patients are depicted in Table 17.1.

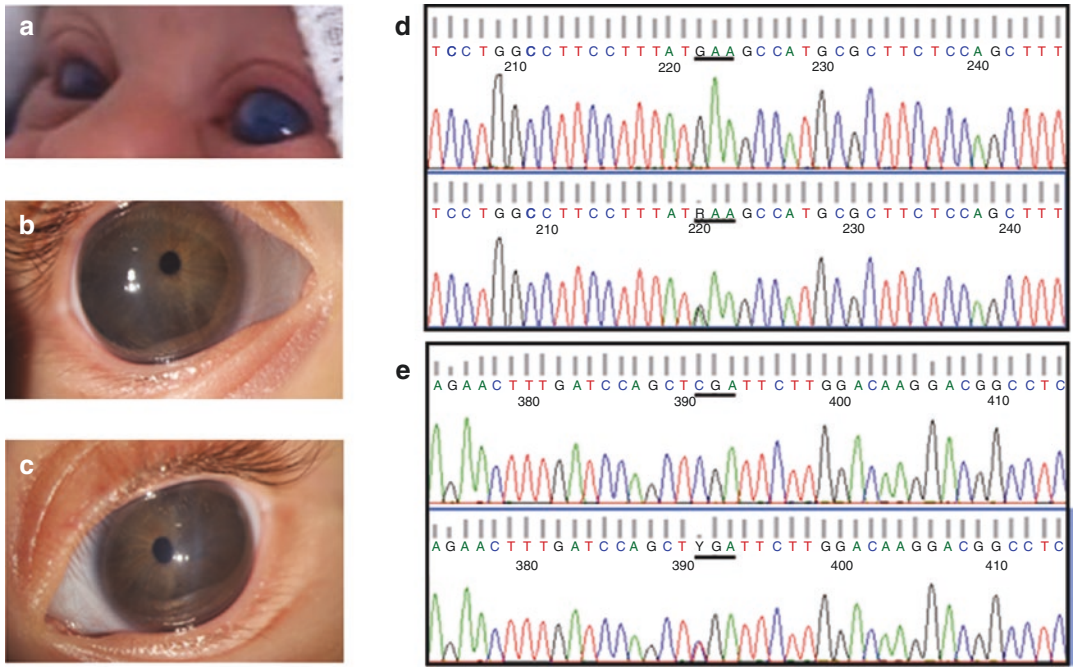

Fig. 17.1 Primary congenital glaucoma patient harboring variants in the *CYP1B1* gene. (**a**) Photograph of the patient at diagnosis showing corneal edema and buphthalmos. (**b**) Left eye after surgeries for IOP reduction. (**c**) Right eye after surgeries for IOP reduction. (**d**) Chromatogram showing the p.E387K variant (GAA-AAA) in heterozygosis. (**e**) Chromatogram showing the p.R444Ter variant (CGA-TGA) in heterozygosis. Photographs are courtesy of Dr. Christiane Rolim de Moura from Federal University of São Paulo

Table 17.1 Distribution of *CYP1B1* mutations associated with PCG observed in Brazilian cohorts

Genomic DNA position	Amino acid change	Allele frequencies (%)	Origin
g.3860C>T	p.Q19Ter	1.14	Brazil [32, 35–37], South Korea [42]
g.3976 G>A	p.W57Ter	1.70	Brazil [32, 36], Australia [43], USA [26], Germany [44], France [45], Hipanic origin [46]
g.3987 G>A	p.G61E	0.28	Brazil [37], Saudi Arabia [21, 23, 47], Iran [48, 49], USA [44], Morocco [50], India [51, 52], Turkey [53], Spain [54], Ecuador [55]
g.4046 T>A	p.Y81N	0.28	Brazil [37], Pakistan [56], Germany [44], Spain [54]
g.4340delG	Frameshift	10.51	Brazil [32, 35, 36], Morocco [50], USA, Hispanic origin [33], North Africa [45]
g.4523delC	Frameshift	0.28	Brazil [35]
g.4635delT	Frameshift	1.70	Brazil [35, 36], Mexico [57]
g.7901_7913delGAGTGCAGGCAGA	Frameshift	3.40	Brazil [32, 35, 36], Turkey [14, 46, 58], France [45], Saudi Arabia [47], USA [26, 44], Russia, Germany, Switzerland [44], Canada [59], Spain [54]
g.7940G>A	p.R368H	0.85	Brazil [32, 36], Saudi Arabia [23], Iran [48, 49], India [36, 51, 52], Turkey [53], Australia [43], USA [44], Pakistan [56], South Korea [42], Germany [44]

Table 17.1 (continued)

Genomic DNA position	Amino acid change	Allele frequencies (%)	Origin
g.7970 T>A	p.L378Q	0.57	Brazil [35, 36]
g.7996 G>A	p.E387K	1.42	Brazil [32, 34–36], Romany [22], France [45], Canada [59], USA [26, 34, 44], Australia [43], Hispanic origin [46]
g.8035 C>T	p.P400S	0.28	Brazil [36], Australia [43], Spain [54]
g.8037_8046dupTCATGCCACC	Frameshift	5.11	Brazil [32, 35–37], France [45], India [36, 51], Turkey [46, 53], USA [44, 46], UK [46], Pakistan [56], Spain [54]
g.8147C>T	p.P437L	1.70	Brazil [32, 36, 37], Turkey [46], India [36, 51], Saudi Arabia [47], Spain [54]
g.8165 C>G	p.A443G	1.14	Brazil [32, 35, 36], Saudi Arabia [47], Ethiopia [60], Lebanon [58], USA [26], India [36]
g.8168 G>A	p.R444Q	0.28	Brazil [36], Japan [61], South Korea [42], Australia [43], France [45]
g.8182delG	Frameshift	2.27	Brazil [32, 35], USA [46], Portugal [45]
g.8214_8215delAG	Frameshift	0.85	Brazil [32, 35, 36], India [36]
g. 8263 T>C	p.S476P	0.28	Brazil, India [36]

As previously reported, the Brazilian population is highly admixed and heterogeneous. It is the result of several immigration events accompanied by the miscegenation of three major ancestral roots: Amerindians, Europeans, and Africans. Genetic composition varies from region to region, but it has been shown that the urban population is more uniform than previously thought. For autosomal markers, the proportion of European, African, and Amerindian ancestries was estimated between 70 and 77%, 13 and 19%, and 9 and 10%, respectively [38–40]. Accordingly, the study by Rolim et al. evaluated ancestry markers in PCG patients from the State of Minas Gerais and reported that the proportion of Europeans, Africans, and Amerindians ranged from 74 to 83%, 11 to 18%, and 4 to 9%, respectively. The authors demonstrated that African ancestry was more frequent in PCG cases than in controls (although with no statistical significance) and that it was associated with a higher number of surgeries to control IOP, suggesting that it might act as risk factor for the disease when in high proportion [41].

17.5 Summary

The studies evaluating the participation of the *CYP1B1* gene as causative for PCG in Brazil have shown a frequency of disease-associated variants ranging from 23.5 to 50.0%. This important contribution strongly suggests that this gene is worth being tested in Brazilian PCG patients. Most of the patients present compound heterozygosity in their genotype, reinforcing the admixture profile of the Brazilian population.

The most frequent disease-associated variants in Brazil are g.4340delG, followed by g.8037_8046dupTCATGCCACC, and g.7901_7913delGAGTGCAGGCAGA. It is important to notice that only one study was performed in the Northeast region, with only 17 PCG patients included. This was enough to identify two new variants in Brazil, which emphasizes the need for additional studies in all regions of Brazil, in order to obtain a more realistic representation of PCG in this population.

Twenty years after the identification of *CPYP1B1* gene, two variants remain exclusively

identified in Brazil: g.4523delC and L378Q. The other variants are shared with several population groups, but four were reported in only one other country: South Korea (p.Q19Ter), Mexico (g.4635delT), and India (g.8214_8215delAG and p.S476P). It would be interesting to evaluate if these four disease-associated variants are originated from a common founder or if they are de novo events.

Brazil can contribute in the understanding of the genetic basis of PCG by searching for new genes using family-based approach, as well as investigating the genes recently associated with PCG, *LTBP2*, and *TEK*. The latter seems to be more promising, since their disease-associated variants appear to be more spread in different populations than *LTBP2* variants. Finally, it is important that more collaborative studies are made to better reveal the genetic basis of PCG and to establish genotype–phenotype correlations applicable in precision medicine. For example, if *TEK* alterations are associated with the absence of Schlemm's canal, the primary angle surgery might not be the ideal surgical treatment option.

References

1. Tamm ER. The trabecular meshwork outflow pathways: structural and functional aspects. Exp Eye Res. 2009;88:648–55.
2. Chang EE, Goldberg JL. Glaucoma 2.0: neuroprotection, neuroregeneration, neuroenhancement. Ophthalmology. 2012;119:979–86.
3. 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;2015:321291. https://doi.org/10.1155/2015/321291.
4. Weinreb RN, Grajewski AL, Papadopoulos M, Grigg J, Freedman S. Childhood Glaucoma: the 9th consensus report of the World Glaucoma Association, Vancouver, BC. Amsterdam: Klugler; 2013.
5. Khan AO. Genetics of primary glaucoma. Curr Opin Ophthalmol. 2011;22:347–55.
6. Lopes NLV, Gracitelli CPB, Moura CR. Creation of a childhood glaucoma registry database. Arq Bras Oftalmol. 2018;81:271–5.
7. de Paula CH, Vasconcelos GC, Nehemy MB, Granet D. Causes of visual impairment in children seen at a university-based hospital low vision service in Brazil. J AAPOS. 2015;19:252–6.
8. Haddad MA, Sei M, Sampaio MW, Kara-José N. Causes of visual impairment in children: a study of 3,210 cases. J Pediatr Ophthalmol Strabismus. 2007;44:232–40.
9. Kong L, Fry M, Al-Samarraie M, Gilbert C, Steinkuller PG. An update on progress and the changing epidemiology of causes of childhood blindness worldwide. J AAPOS. 2012;16:501–7.
10. Ko F, Papadopoulos M, Khaw PT. Primary congenital glaucoma. Prog Brain Res. 2015;221:177–89.
11. Lewis CJ, Hedberg-Buenz A, DeLuca AP, Stone EM, Alward WLM, Fingert JH. Primary congenital and developmental glaucomas. Hum Mol Genet. 2017;26(R1):R28–36. https://doi.org/10.1093/hmg/ddx205.
12. Online Mendelian inheritance in man. https://www.ncbi.nlm.nih.gov/omim/?term=CYP1B1
13. Sarfarazi M, Akarsu AN, Hossain A, Turacli ME, Aktan SG, Barsoum-Homsy M, et al. Assignment of a locus (GLC3A) for primary congenital glaucoma (Buphthalmos) to 2p21 and evidence for genetic heterogeneity. Genomics. 1995;30(2):171–7.
14. Stoilov I, Akarsu AN, Sarfarazi M. 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. Hum Mol Genet. 1997;6:641–7.
15. Shimada T, Hayes CL, Yamazaki H, Amin S, Hecht SS, Guengerich FP, et al. Activation of chemically diverse procarcinogens by human cytochrome P-450 1B1. Cancer Res. 1996;56:2979–84.
16. Stoilov I, Jansson I, Sarfarazi M, Schenkman JB. Roles of cytochrome p450 in development. Drug Metabol Drug Interact. 2001;18:33–55.
17. Choudhary D, Jansson I, Schenkman JB. CYP1B1, a developmental gene with a potential role in glaucoma therapy. Xenobiotica. 2009;39:606–15.
18. Zhao Y, Sorenson C, Sheibani N. Cytochrome P4501B1 and primary congenital glaucoma. J Ophthalmic Vis Res. 2015;10:60–7.
19. Tang YM, Wo YY, Stewart J, Hawkins AL, Griffin CA, Sutter TR, Greenlee WF. Isolation and characterization of the human cytochrome P450 CYP1B1 gene. J Biol Chem. 1996;271:28324–30.
20. The Human gene mutation database. http://www.hgmd.cf.ac.uk/ac/gene.php?gene=CYP1B1
21. Bejjani BA, Lewis RA, Tomey KF, Anderson KL, Dueker DK, Jabak M, et al. Mutations in CYP1B1, the gene for cytochrome P4501B1, are the predominant cause of primary congenital glaucoma in Saudi Arabia. Am J Hum Genet. 1998;62:325–33.
22. Plásilová M, Stoilov I, Sarfarazi M, Kádasi L, Feráková E, Ferák V. Identification of a single ancestral CYP1B1 mutation in Slovak Gypsies (Roms) affected with primary congenital glaucoma. J Med Genet. 1999;36:290–4.
23. Bejjani BA, Stockton DW, Lewis RA, Tomey KF, Dueker DK, Jabak M, et al. Multiple CYP1B1 muta-

tions 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.
24. Yang M, Guo X, Liu X, Shen H, Jia X, Xiao X, Li S, Fang S, Zhang Q. Investigation of CYP1B1 mutations in Chinese patients with primary congenital glaucoma. Mol Vis. 2009;15:432–7.
25. Fuse N, Miyazawa A, Takahashi K, Noro M, Nakazawa T, Nishida K. Mutation spectrum of the CYP1B1 gene for congenital glaucoma in the Japanese population. Jpn J Ophthalmol. 2010;54:1–6.
26. Lim SH, Tran-Viet KN, Yanovitch TL, Freedman SF, Klemm T, Call W, et al. CYP1B1, MYOC, and LTBP2 mutations in primary congenital glaucoma patients in the United States. Am J Ophthalmol. 2013;155:508–17.e5.
27. Li N, Zhou Y, Du L, Wei M, Chen X. Overview of Cytochrome P4501B1 gene mutations in patients with primary congenital glaucoma. Exp Eye Res. 2011;93:572–9.
28. Akarsu AN, Turacli ME, Aktan SG, Barsoum-Homsy M, Chevrette L, Sayli BS, et al. A second locus (GLC3B) for primary congenital glaucoma (Buphthalmos) maps to the 1p36 region. Hum Mol Genet. 1996;5:1199–203.
29. Stoilov, I. R., Sarfarazi, M. The third genetic locus (GLC3C) for primary congenital glaucoma (PCG) maps to chromosome 14q24.3 (Abstract). Invest Ophthal Vis Sci. 2002;43:e3015.
30. Ali M, McKibbin M, Booth A, Parry DA, Jain P, Riazuddin SA, et al. Null mutations in LTBP2 cause primary congenital glaucoma. Am J Hum Genet. 2009;84:664–71.
31. Souma T, Tompson SW, Thomson BR, Siggs OM, Kizhatil K, Yamaguchi S, et al. Angiopoietin receptor TEK mutations underlie primary congenital glaucoma with variable expressivity. J Clin Invest. 2016;126:2575–87.
32. Stoilov IR, Costa VP, Vasconcellos JP, Melo MB, Betinjane AJ, Carani JC, et al. Molecular genetics of primary congenital glaucoma in Brazil. Invest Ophthalmol Vis Sci. 2002;43:1820–7.
33. Hollander DA, Sarfarazi M, Stoilov I, Wood IS, Fredrick DR, Alvarado JA. Genotype and phenotype correlations in congenital glaucoma. Trans Am Ophthalmol Soc. 2006;104:183–95.
34. Sena DF, Finzi S, Rodgers K, Del Bono E, Haines JL, Wiggs JL. Founder mutations of CYP1B1 gene in patients with congenital glaucoma from the United States and Brazil. J Med Genet. 2004;41(1):e6.
35. Della Paolera M, de Vasconcellos JP, Umbelino CC, Kasahara N, Rocha MN, Richeti F, et al. CYP1B1 gene analysis in primary congenital glaucoma Brazilian patients: novel mutations and association with poor prognosis. J Glaucoma. 2010;19:176–82.
36. Melo MB, Mandal AK, Tavares IM, Ali MH, Kabra M, de Vasconcellos JP, et al. Genotype-phenotype correlations in CYP1B1-associated primary congenital glaucoma patients representing two large cohorts from India and Brazil. PLoS One. 2015;10(5):e0127147. https://doi.org/10.1371/journal.pone.0127147.
37. Coêlho REA, Sena DR, Santa Cruz F, Moura BCFS, Han CC, Andrade FN, et al. CYP1B1 gene and phenotypic correlation in patients from Northeastern Brazil with primary congenital glaucoma. J Glaucoma. 2019;28:161–4.
38. Giolo SR, Soler JM, Greenway SC, Almeida MA, de Andrade M, Seidman JG, et al. Brazilian urban population genetic structure reveals a high degree of admixture. Eur J Hum Genet. 2012;20:111–6.
39. Kehdy FS, Gouveia MH, Machado M, Magalhães WC, Horimoto AR, Horta BL, et al. Origin and dynamics of admixture in Brazilians and its effect on the pattern of deleterious mutations. Proc Natl Acad Sci U S A. 2015;112:8696–701.
40. Pena SD, Di Pietro G, Fuchshuber-Moraes M, Genro JP, Hutz MH, Kehdy F de S, et al. The genomic ancestry of individuals from different geographical regions of Brazil is more uniform than expected. PLoS One. 2011;6(2):e17063. https://doi.org/10.1371/journal.pone.0017063.
41. Rolim H, Cronemberger S, Rangel H, Batista WD, Bastos-Rodrigues L, De Marco L. The role of genetic ancestry in Brazilian patients with primary congenital glaucoma. J Glaucoma. 2016;25:24–8.
42. Suh W, Kee C. A clinical and molecular genetics study of primary congenital glaucoma in South Korea. Br J Ophthalmol. 2012;96:1372–7.
43. Dimasi DP, Hewitt AW, Straga T, Pater J, MacKinnon JR, Elder JE, et al. Prevalence of CYP1B1 mutations in Australian patients with primary congenital glaucoma. Clin Genet. 2007;72:255–60.
44. Chavarria-Soley G, Michels-Rautenstrauss K, Pasutto F, Flikier D, Flikier P, Cirak S, et al. Primary congenital glaucoma and Rieger's anomaly: extended haplotypes reveal founder effects for eight distinct CYP1B1 mutations. Mol Vis. 2006;12:523–31.
45. Colomb E, Kaplan J, Garchon HJ. Novel cytochrome P450 1B1 (CYP1B1) mutations in patients with primary congenital glaucoma in France. Hum Mutat. 2003;22:496.
46. Stoilov I, Akarsu AN, Alozie I, Child A, Barsoum-Homsy M, Turacli ME, et al. 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. Am J Hum Genet. 1998;62:573–84.
47. Abu-Amero KK, Osman EA, Mousa A, Wheeler J, Whigham B, Allingham RR, et al. Screening of CYP1B1 and LTBP2 genes in Saudi families with primary congenital glaucoma: genotype-phenotype correlation. Mol Vis. 2011;17:2911–9.
48. Chitsazian F, Tusi BK, Elahi E, Saroei HA, Sanati MH, Yazdani S, et al. CYP1B1 mutation profile of Iranian primary congenital glaucoma patients and associated haplotypes. J Mol Diagn. 2007;9:382–93.

49. Suri F, Yazdani S, Narooie-Nejhad M, Zargar SJ, Paylakhi SH, Zeinali S, et al. Variable expressivity and high penetrance of CYP1B1 mutations associated with primary congenital glaucoma. Ophthalmology. 2009;116:2101–9.
50. Belmouden A, Melki R, Hamdani M, Zaghloul K, Amraoui A, Nadifi S, et al. A novel frameshift founder mutation in the cytochrome P450 1B1 (CYP1B1) gene is associated with primary congenital glaucoma in Morocco. Clin Genet. 2002;62:334–9.
51. Reddy AB, Kaur K, Mandal AK, Panicker SG, Thomas R, Hasnain SE, et al. Mutation spectrum of the CYP1B1 gene in Indian primary congenital glaucoma patients. Mol Vis. 2004;10:696–702.
52. Panicker SG, Mandal AK, Reddy AB, Gothwal VK, Hasnain SE. Correlations of genotype with phenotype in Indian patients with primary congenital glaucoma. Invest Ophthalmol Vis Sci. 2004;45:1149–56.
53. Bagiyeva S, Marfany G, Gonzalez-Angulo O, Gonzalez-Duarte R. Mutational screening of CYP1B1 in Turkish PCG families and functional analyses of newly detected mutations. Mol Vis. 2007;13:1458–68.
54. Campos-Mollo E, López-Garrido MP, Blanco-Marchite C, Garcia-Feijoo J, Peralta J, Belmonte-Martínez J, et al. CYP1B1 mutations in Spanish patients with primary congenital glaucoma: phenotypic and functional variability. Mol Vis. 2009;15:417–31.
55. Curry SM, Daou AG, Hermanns P, Molinari A, Lewis RA, Bejjani BA. Cytochrome P4501B1 mutations cause only part of primary congenital glaucoma in Ecuador. Ophthalmic Genet. 2004;25:3–9.
56. Rauf B, Irum B, Kabir F, Firasat S, Naeem MA, Khan SN, et al. A spectrum of CYP1B1 mutations associated with primary congenital glaucoma in families of Pakistani descent. Hum Genome Var. 2016;3:16021. https://doi.org/10.1038/hgv.2016.21.
57. Messina-Baas OM, González-Huerta LM, Chima-Galán C, Kofman-Alfaro SH, Rivera-Vega MR, Babayán-Mena I, et al. Molecular analysis of the CYP1B1 gene: identification of novel truncating mutations in patients with primary congenital glaucoma. Ophthalmic Res. 2007;39:17–23.
58. Michels-Rautenstrauss KG, Mardin CY, Zenker M, Jordan N, Gusek-Schneider GC, Rautenstrauss BW. Primary congenital glaucoma: three case reports on novel mutations and combinations of mutations in the GLC3A (CYP1B1) gene. J Glaucoma. 2001;10:354–7.
59. Martin SN, Sutherland J, Levin AV, Klose R, Priston M, Héon E. Molecular characterisation of congenital glaucoma in a consanguineous Canadian community: a step towards preventing glaucoma related blindness. J Med Genet. 2000;37:422–7.
60. Aklillu E, Oscarson M, Hidestrand M, Leidvik B, Otter C, Ingelman-Sundberg M. Functional analysis of six different polymorphic CYP1B1 enzyme variants found in an Ethiopian population. Mol Pharmacol. 2002;61:586–94.
61. Mashima Y, Suzuki Y, Sergeev Y, Ohtake Y, Tanino T, Kimura I, et al. Novel cytochrome P4501B1 (CYP1B1) gene mutations in Japanese patients with primary congenital glaucoma. Invest Ophthalmol Vis Sci. 2001;42:2211–6. Erratum in: Invest Ophthalmol Vis Sci. 2001;42:2775.