• Basic Research •

Compound heterozygous mutations in *CYP1B1* gene leads to severe primary congenital glaucoma phenotype

Na Song[1], Lin Leng[1], Xue-Jiao Yang[1], Yu-Qing Zhang[1], Chun Tang[1], Wen-Shi Chen[1], Wei Zhu[2], Xian Yang[1]

[1]Department of Ophthalmology, the Affiliated Hospital of Qingdao University, Qingdao 266003, Shandong Province, China
[2]Department of Pharmacology, School of Pharmacy, Qingdao University, Qingdao 266021, Shandong Province, China
Correspondence to: Xian Yang. Department of Ophthalmology, the Affiliated Hospital of Qingdao University, Qingdao 266003, Shandong Province, China. yangxian_zhao@126.com; Wei Zhu. Department of Pharmacology, School of Pharmacy, Qingdao University, Qingdao 266021, Shandong Province, China. wzhu@qdu.edu.cn
Received: 2018-09-20 Accepted: 2018-12-15

Abstract

• AIM: To identify the novel mutation alleles in the *CYP1B1* gene of primary congenital glaucoma (PCG) patients at Shandong Province of China, and investigate their correlation with glaucomatous features.

• METHODS: The DNA from the peripheral blood of 13 congenital glaucoma patients and 50 ethnically matched healthy controls from the affiliated hospital of Qingdao University were extracted. The coding region of the *CYP1B1* gene was amplified by PCR and direct DNA sequencing was performed. Disease causing-variants were analyzed by comparing the sequences and the structures of wild type and mutant *CYP1B1* proteins by PyMOL software.

• RESULTS: Two missense mutations, including A330F caused by c.988G>T&c.989C>T, and R390H caused by c.1169G>A, were identified in one of the 13 PCG patients analyzed in our study. A330F mutation was observed to be novel in the Chinese Han population, which dramatically altered the protein structure of *CYP1B1* gene, including the changes in the ligand-binding pocket. Furthermore, R390H mutation caused the changes in heme-protein binding site of this gene. In addition, the clinical phenotype displayed by PCG patient with these mutations was more pronounced than other PCG patients without these mutations. Multiple surgeries and combined drug treatment were not effective in reducing the elevated intraocular pressure in this patient.

• CONCLUSION: A novel A330F mutation is identified in the *CYP1B1* gene of Chinese PCG patient. Moreover, in combination with other mutation R390H, this PCG patient shows significant difference in the *CYP1B1* protein structure, which may specifically contribute to severe glaucomatous phenotype.

• KEYWORDS: primary congenital glaucoma; *CYP1B1* gene; missense mutation; protein structure

DOI:10.18240/ijo.2019.06.05

Citation: Song N, Leng L, Yang XJ, Zhang YQ, Tang C, Chen WS, Zhu W, Yang X. Compound heterozygous mutations in *CYP1B1* gene leads to severe primary congenital glaucoma phenotype. *Int J Ophthalmol* 2019;12(6):909-914

INTRODUCTION

Primary congenital glaucoma (PCG), alternatively known as developmental glaucoma, is a developmental eye defect, primarily caused by the abnormal development of anterior chamber angle during neonatal or infantile stage[1]. Typically, most patients display glaucomatous phenotypes during birth, but in some cases its onset has also been observed at adolescence[2]. Typical symptoms of PCG include tearing, photophobia, and blepharospasm[3]. In absence of any treatment, ocular hypertension can lead to irreversible damage to the optic nerve, thereby resulting in reduced vision or subsequent complete blindness.

Congenital glaucoma has been shown to be a hereditary disease transmitted in an autosomal recessive pattern[4]. Although it's complete etiology has not been thoroughly elucidated, genetic cause is believed to be the most important risk factor in PCG patients. Since the first genetic study in PCG patients[5], multiple genetic loci in the gene *GLC3* have been identified, including *GLC3A*[5], *GLC3B*[6], *GLC3C*, and *GLC3D*[7]. These studies have shown the presence of two pathogenic genes, *cytochrome P450 1B1* (*CYP1B1*) and *latent-transforming growth factor beta-binding protein 2* (*LTBP2*) on these genetic loci. The mutation in the *CYP1B1* gene is considered as the most common pathological reason for congenital glaucoma. Typically, CYP1B1 protein structure display 4 conserved helix bundles, including J-helix, K-helix, β-sheets, meander region, and heme-binding region[8]. In addition, the N-terminus hinge region and C-terminus conserved core structures (CCSs) have

been reported to be the most crucial regions for maintaining the fundamental properties of *CYP1B1*[2,9]. Interestingly, the clinical reports from different ethnic groups showed that PCG patients, with *CYP1B1* mutations, like missense mutations of W57C[9] and G365W[10] in the N-terminus hinge region or CCSs, generally have more severe glaucomatous phenotypes. To date, more than 150 mutation variants of *CYP1B1* gene have been found in congenital glaucoma patients worldwide[11]. In Chinese patients, 43 mutation variants have been reported, including R390H, the common mutation identified in PCG patients from all ethnic groups, and L107V mutations uniquely identified in Chinese PCG patients only[12].

Based on this information, we in our study have tried to investigate if any novel *CYP1B1* gene mutations exist in PCG patients from Shandong province of China. To address it, we analyzed the *CYP1B1* gene individually from 13 PCG diagnosed Chinese patients, and 50 healthy controls from Shandong Province by direct sequencing of its coding region. The structure of the mutated protein was analyzed through PyMOL software (USA). The severe glaucomatous phenotype of one PCG patient with novel mutation indicated the important role played by specific mutational allele in regulating *CYP1B1* gene function during development.

SUBJECTS AND METHODS

Ethical Approval The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Affiliated Hospital of Qingdao University. The informed consents were obtained from each subject or his/her guardian for their participation in this study.

The ocular examination of the sporadic PCG patients and ethnically matched healthy controls were performed at Affiliated Hospital of Qingdao University. The examination included vision tests, intraocular pressure (IOP), slit lamp bio-microscopy, corneal diameter, and cup-to-disc ratio. Based on these clinical reports, patients with other ocular diseases beside PCG or systemic illnesses were excluded from this study. Based on the IOP, 13 sporadic PCG patients with IOP of higher than 21 mm Hg, and 50 healthy controls with IOP lower than 21 mm Hg, were eventually selected for this study.

Peripheral blood samples were collected from all subjects. Leukocyte DNA were extracted using Massive Whole Blood Genomic DNA Extraction Kit (BioTeke, Beijing, China). The coding region of *CYP1B1* gene was amplified from the genomic DNA by polymerase chain reaction (PCR). The PCR reaction was performed in a 25 μL reaction mixture containing 0.1 μg genomic DNA, 40 μmol/L forward and reverse primers (Table 1), 3 mmol/L magnesium chloride and 2×Hieff™ PCR Master Mix (YESEN Biotech Co., Shanghai, China). The following PCR conditions were used for amplification: an initial denaturation step at 95℃ for 5min, 32 cycles of denaturation at 95℃ for 30s, annealing temperature for 30s, and extension at 72℃ for 30s, with final additional extension time of 10min at 72℃.

Table 1 The primers to amplify coding regions of *CYP1B1* gene

Name	Sequence (5' to 3')	Size of the products (bp)
CYP1B1-2F	TCAGCTCCGACCTCTCCACCCA	1316
CYP1B1-2R	AGTCCCTTTACCGACGCGATCT	
CYP1B1-3F	TTCTTA AAGTCCATCTTGTAAT	997
CYP1B1-3R	AAAAAAATCTCCCAGAAGCTCC	

CYP1B1: Cytochrome P450 Family 1 Subfamily B Member 1.

The amplified PCR products were subjected to agarose gel electrophoresis and the target PCR fragments were extracted from Biowest Agarose by QIAquick Gel Extraction Kit (QIAGEN, Shanghai, China). Next, the direct sequencing of the amplified product was completed using amplification primers (Ruibo Xingke Biotech Co. Beijing, China), and the sequences were analyzed using Chromas V2.3, DNAman V7.0 software along with PubMed Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi) search engine.

In addition, the sequence of wild-type *CYP1B1* gene was downloaded from https://www.rcsb.org, and saved as PDB file. The mutated amino acids in the *CYP1B1* protein were changed manually using ICMpro software, and the PDB files were uploaded into PyMOL software to generate 3D structure of the protein. The mutated amino acids and key ligands were labeled in different colors.

RESULTS

Among the total 13 primary congenital glaucoma patients included in our study, 12 were males and 1 female, with age ranging from 3 to 23y. The median age at the time of diagnosis was 3mo. In addition, one patient had unilateral ocular abnormality diagnosis, while all other 12 patients were diagnosed with bilateral congenital glaucoma. The average IOP prior to surgeries in these patients was 37.19 mm Hg and the mean corneal diameter was 13 mm.

The analysis of *CYP1B1* gene led to the identification of two missense mutations in one of the 13 PCG patients through direct sequence analysis, including c.988G>T [G=0.00000 (0/112670, ExAC)] and c.989C>T [A=0.00000 (1/244488, GnomAD)] (P.A330F) and c.1169G>A [T=0.00009 (22/244260, GnomAD)] (P.R390H) (Figure 1). These variants were not detected in any of the 50 healthy controls. In comparison to the previous studies, R390H mutation was common in Chinese PCG patients[13], but A330F mutation was novel in Chinese Han population. It is also important to highlight that it is for the first time that these two mutations were identified in single PCG patient worldwide. Furthermore, in addition to these two missense mutations, four other single-nucleotide

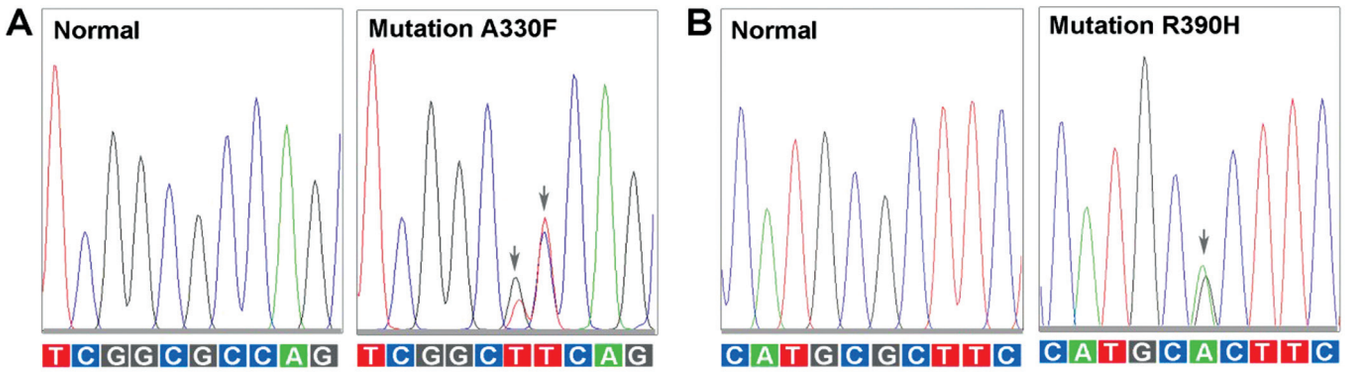

Figure 1 Electropherograms of two mutations in the *CYP1B1* gene The normal and the mutated nucleic acids from region 983 to 992 (A); and region 1164 to 1173 (B) of *CYP1B1* gene have been listed. The mutated nucleic acids are labeled with arrows.

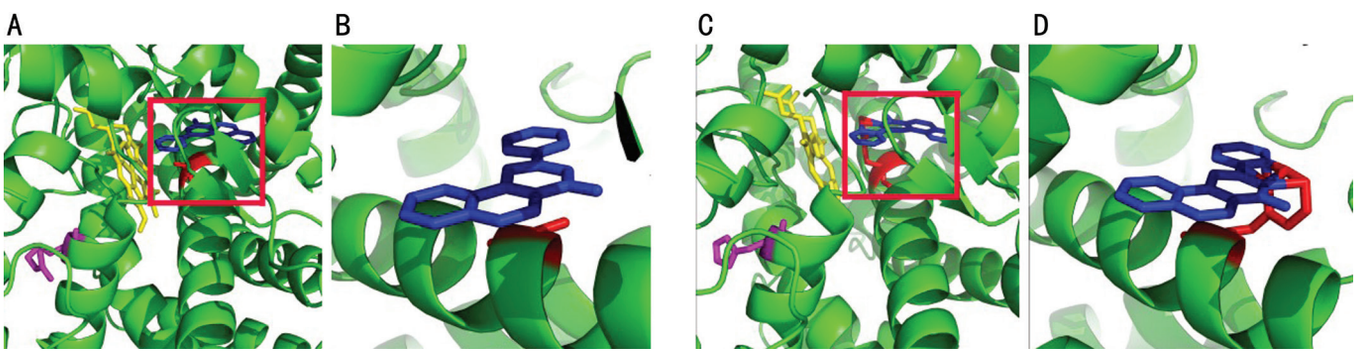

Figure 2 The structural analysis of wild-type and mutated CYP1B1 protein A: The normal CYP1B1 protein structure including amino acid, alanine (labeled as red) at position 330 and arginine (labeled as purple) at position 390. The ANF ligand and HEM (Protoporphyrin Ix Containing Fe) ligand have been labeled as blue and yellow, respectively. B: The interaction between alanine and ANF ligand under high magnification. C, D: The influence of the mutations on the interactions between amino acid and ligands.

polymorphisms (SNPs) were also identified in other analyzed PCG patients or healthy controls, including c.142C>G (P.R48W), c.355G>T (P.A119S), c.1347T>C (P.D449D), and c.1294C>G (P.L432V).

Next, to predict the functional correlation of these mutations in the *CYP1B1* gene, protein structure was analyzed using PyMOL software. As shown in Figure 2, A330F and R390H mutations disrupted the orientation of I-helix and K-helix, respectively. More specifically, the link between the substrate binding region and the CCS, the torsion of I-helix switched lactamine into phenylalanine in the side chain of the protein. The benzene ring in the phenylalanine can form the connections with the α-naphthoflavone (ANF) ligand, and thus can create stearic hindrance between ANF ligand and heme iron. In addition, another mutation R390H changed K-helix in CCS, and resulted in the formation of unstable heme-protein complex. Together, these findings indicated that both A330F and R390H were important alleles to maintain the fundamental properties of *CYP1B1* gene, and might contribute to severe clinical phenotype.

Interestingly, our clinical findings confirmed our assumption that PCG patient with A330F and R390H mutations exhibited severe glaucoma phenotype. This patient specifically had PCG

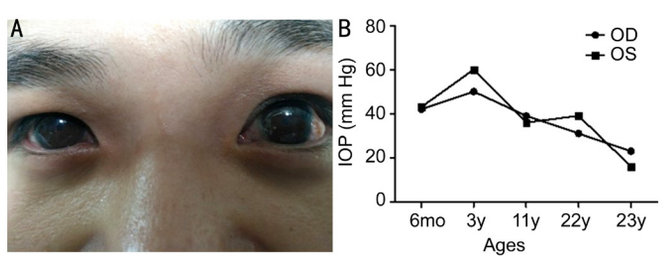

Figure 3 The clinical characteristics of PCG patient with A330F and R390F mutations A: The appearance of the eyes at 23 years of age; B: Information about the changes in IOPs after different treatments and ages.

symptoms at birth with photophobia, tearing, and corneal whitening. However, no family history of PCG has been identified in this patient. As shown in Figure 3, the IOPs in the right and left eye of this patient were 42 and 43 mm Hg, respectively, at 6-month of age, and both horizontal corneal diameters were 12 mm. Goniotomy was performed on both eyes of this patients at Linyi People's Hospital, and after the surgery, the reduced IOPs were maintained for 6mo, but then gradually increased. At the age of 3 years, the IOP of both eyes reached up to 50-60 mm Hg. Subsequently, after trabeculotomy, the postoperative IOPs were again reduced, and maintained for only another 6mo. After the patient turned

11-years-old, the corrected visions were only 0.07 and 0.05 and the IOPs of his right and left eye reached to 39 and 36 mm Hg, respectively. Goniotomy along with trabeculotomy was again performed to reduce his IOPs. Surprisingly, the elevated IOPs could not be reduced effectively this time. Later glaucoma valve was implanted to his left eye in the affiliated hospital of Qingdao University, which then led to reduced IOPs of 16 mm Hg. Next, with the help of betamethanil eye drops, the IOPs were stabilized at 18-23 mm Hg for the right eye, and 14-16 mm Hg for the left eye. More recently, the corrected vision of the right eye was 0.05, and for the left eye, it was finger counting (10 cm), with corneal diameter of 13 mm, for both eyes. The structures behind the iris could not be observed by slit lamp, due to the adherence of iris in the pupil area to mildly opaque lens.

DISCUSSION

The congenital glaucoma has mainly been characterized as genetically inherited disease, and specifically *CYP1B1* gene mutations show association with its pathogenesis[14-17]. Importantly, more than 150 mutations in the *CYP1B1* gene have been reported to be associated with PCG pathophysiology, but recently specific *CYP1B1* mutations have been predominantly linked with severe glaucomatous phenotypes[18]. Our study detected common variant, R390H in the *CYP1B1* gene among Chinese PCG patient. This mutation has been reported in different ethnic groups worldwide[19-20], and has been shown to cause severe angle abnormalities. In addition, our study also demonstrated the presence of another novel variant, A330F among the same Chinese PCG patient. However, this additional variant has previously been only reported in Japanese[21] and South Korean[22]. According to the SNP database in NCBI (https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=1545), the mutant allele frequency of A330F is much lower than the frequency in R390H. The presence of A330F in limited number of PCG patients in Asia suggest that it is a different allele, capable of inducing severe PCG phenotypes. Up until this report, no evidence existed about the co-occurrence of both these two missense mutations, R390H and A330F, in same congenital glaucoma patient. The Chinese PCG patient in our study was indeed the first one to show this trend in the world. Further clinical observation confirmed the correlation between the presence of these mutations and patient displaying severe glaucomatous phenotypes. Multiple surgeries and combined medicinal treatment was required to reduce the elevated IOP in this patient.

As the functional importance of *CYP1B1* can be predicted through its protein structure analysis[23], it can act as an excellent strategy to understand the correlation between specific mutations and severe angle abnormalities. The carboxyl terminus of *CYP1B1* protein includes a substrate binding region and CCS, while the N-terminal of *CYP1B1* protein includes a membrane-spanning domain and a hinge region. Generally, the critical mutations in *CYP1B1* gene has been located in the hinge region and CCS[2]. The critical mutation, R390H observed in our study usually disrupt the orientation of K-helix in CCS, which then affects the formation of a stable haemoglobin complex and molecular folding[9,24]. This mutation clearly changes the fundamental features of *CYP1B1*, and can lead to severe developmental abnormalities as reported previously[22]. Another mutation detected in the *CYP1B1* gene in PCG patient in our study was A330F, which is located in the I-helix and provide link between substrate binding region and CCS. Historically, the mutation in this region is not as common as R390H, and thus has not been previously considered very crucial in PCG patients. However, recently more and more numbers of PCG patients have confirmed the close correlations between severe phenotypes and this mutation. In addition, mutations like E229K[25] and S239R[26], have also been shown to involved in the disruption of the three-dimensional structures of I-helix, and subsequently lead to severe glaucomatous phenotypes. Moreover, consistent with earlier analysis, our structural analysis also suggested that the mutation in the I-helix can block binding of substrates with heme iron. Altogether, these cumulative evidences indicate that the link region is also an equally important domain for maintaining the function of *CYP1B1* gene, besides the hinge region and CCS.

The *CYP1B1* enzyme has been shown to play an important and crucial catalytic role in the cholesterol, steroids, and other lipids synthesis[27-29]. All these metabolic reactions and products seem to be important in the differentiation and growth of multiple tissues. In the context of PCG, *CYP1B1* mutations cause significant change in retinol, which is the key metabolite for the development of trabecular meshwork (TM)[30-31]. Multiple studies have indicated about the important roles of retinol in the TM development; like mutations in the retinol receptor gene and the retinoic acid receptor β cause severe developmental defects[32]. The retinoic acid receptors and retinoid X receptors expressed in the TM can transform retinol into its active derivatives which then regulate the transcription of myocilin gene[29,33]. In addition, the function of the *CYP1B1* gene in regulating aqueous humor outflow is controversial, but glaucoma pathology is highly related with the mutation and expression of this gene. In our study, we speculate that heme instability caused by R390H and A330F mutations can lead to the abnormalities in retinol metabolism, and is supported by the functional studies published in recent years[30]. Collectively, the evidence indicates that the retinol metabolic abnormalities due to R390H and A330F mutation might contribute to severe glaucomatous phenotypes in PCG patients. Alternatively,

another major cause of TM pathogenesis can be abnormal oxidation status due to abnormal *CYP1B1* gene activity. Presence of oxidative stress during early development can cause TM hypoplasia, which then can lead to PCG development[34]. It has been specifically reported that antioxidant enzymes are deficient in the TM of PCG patient, and the presence of high H_2O_2 and superoxide anions in the aqueous humour can easily facilitate their infiltration into the TM, and can subsequently exert oxidative stress, eventually leading to TM pathogenesis[35].

Currently, goniotomy is the major approach to treat congenital glaucoma. Although the single surgery can benefit certain PCG patients, but in some patients with specific mutations in *CYP1B1* genes, repetitive surgeries combined with other medicine are required for treatment. But the combined treatments are also not effective to reduce IOP in these patients, and therefore, it is necessary to explore novel approaches for treatment of PCG patients, especially for the patients like in our study with multiple *CYP1B1* mutations. In this direction, a recent study reported that abnormal cellular function of TM cells from *CYP1B1*$^{-/-}$ mouse can be restored by the treatment with the free radical scavenger, N-acetylcysteine[34]. Similarly, supplementing antioxidant enzymes or/and the retinol to the TM, can also probably benefit PCG patients with A330F and R390H mutations. In addition, the CRISPR-Cas9 based gene therapy and stem cells-based therapy have also been reported as promising approaches to rescue the glaucomatous phenotypes for open-angle glaucoma through rebuilding damaged TM[36-37]. Thus, it would be interesting to test these approaches in PCG patients.

Overall, our study demonstrated the presence of novel mutations in the Chinese PCG patients. This is the first report about co-existence of A330F and R390H mutations in the same PCG patient. Furthermore, the structural changes caused by these two mutations renewed our current understanding about the role of key domains of *CYP1B1* gene in influencing congenital glaucoma phenotype. It is evident that besides CCS domain, additional regions can also affect the structure of CCS, and are crucial in regulating the function of *CYP1B1* in terms of causing TM abnormalities. However, future studies are required to determine the major influence of these mutations on metabolism and selection of key metabolites, which can probably lead to the development of potential strategies for PCG therapy.

ACKNOWLEDGEMENTS

We thank Dr. Ke-Wei Wang of Qingdao University and Dr. Li-Ning Lu of Tsinghua University to support the protein structural analysis study.

Foundation: Supported by "Clinical medical+X" Project from Department of Medicine of Qingdao University.

Conflicts of Interest: Song N, None; **Leng L,** None; **Yang XJ,** None; **Zhang YQ,** None; **Tang C,** None; **Chen WS,** None; **Zhu W,** None; **Yang X,** None.

REFERENCES

1 deLuise VP, Anderson DR. Primary infantile glaucoma (congenital glaucoma). *Surv Ophthalmol* 1983;28(1):1-19.

2 Zhao Y, Sorenson CM, Sheibani N. Cytochrome P450 1B1 and primary congenital glaucoma. *J Ophthalmic Vis Res* 2015;10(1):60-67.

3 Bouyacoub Y, Ben Yahia S, Abroug N, Kahloun R, Kefi R, Khairallah M, Abdelhak S. CYP1B1 gene mutations causing primary congenital glaucoma in Tunisia. *Ann Hum Genet* 2014;78(4):255-263.

4 Faiq M, Sharma R, Dada R, Mohanty K, Saluja D, Dada T. Genetic, biochemical and clinical insights into primary congenital glaucoma. *J Curr Glaucoma Pract* 2013;7(2):66-84.

5 Sarfarazi M, Akarsu AN, Hossain A, Turacli ME, Aktan SG, Barsoum-Homsy M, Chevrette L, Sayli BS. Assignment of a locus (GLC3A) for primary congenital glaucoma (Buphthalmos) to 2p21 and evidence for genetic heterogeneity. *Genomics* 1995;30(2):171-177.

6 Akarsu AN, Turacli ME, Aktan SG, Barsoum-Homsy M, Chevrette L, Sayli BS, Sarfarazi M. A second locus (GLC3B) for primary congenital glaucoma (Buphthalmos) maps to the 1p36 region. *Hum Mol Genet* 1996;5(8):1199-1203.

7 Narooie-Nejad M, Paylakhi SH, Shojaee S, Fazlali Z, Rezaei Kanavi M, Nilforushan N, Yazdani S, Babrzadeh F, Suri F, Ronaghi M, Elahi E, Paisán-Ruiz C. Loss of function mutations in the gene encoding latent transforming growth factor beta binding protein 2, LTBP2, cause primary congenital glaucoma. *Hum Mol Genet* 2009;18(20):3969-3977.

8 Wang A, Savas U, Stout CD, Johnson EF. Structural characterization of the complex between α-naphthoflavone and human cytochrome P450 1B1. *J Biol Chem* 2011;286(7):5736-5743.

9 Stoilov I, Akarsu AN, Alozie I, Child A, Barsoum-Homsy M, Turacli ME, Or M, Lewis RA, Ozdemir N, Brice G, Aktan SG, Chevrette L, Coca-Prados M, Sarfarazi M. 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(3):573-584.

10 Hollander DA, Sarfarazi M, Stoilov I, Wood IS, Fredrick DR, Alvarado JA. Genotype and phenotype correlations in congenital glaucoma. *Trans Am Ophthalmol Soc* 2006;104:183-195.

11 Li N, Zhou Y, Du L, Wei M, Chen X. Overview of cytochrome P450 1B1 gene mutations in patients with primary congenital glaucoma. *Exp Eye Res* 2011;93(5):572-579.

12 Yuan R, Zhao J, Zhao Y, Ma X, Dai Q, Wang B. Systematic review of CYP1B1 gene mutations in patients with primary congenital glaucoma in China. *J Evid Based Med* 2015;1:42-47.

13 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;15:432-437.

14 Faiq M, Mohanty K, Dada R, Dada T. Molecular diagnostics and genetic counseling in primary congenital glaucoma. *J Curr Glaucoma Pract* 2013;7(1):25-35.

15 Della Paolera M, de Vasconcellos JP, Umbelino CC, Kasahara N, Rocha MN, Richeti F, Costa VP, Tavares A, de Melo MB. CYP1B1 gene analysis in primary congenital glaucoma Brazilian patients: novel mutations and association with poor prognosis. *J Glaucoma* 2010;19(3):176-182.

16 Chouiter L, Nadifi S. Analysis of CYP1B1 gene mutations in patients with primary congenital glaucoma. *J Pediatr Genet* 2017;6(4):205-214.

17 Sitorus R, Ardjo SM, Lorenz B, Preising M. CYP1B1 gene analysis in primary congenital glaucoma in Indonesian and European patients. *J Med Genet* 2003;40(1):e9.

18 Chen Y, Jiang D, Yu L, Katz B, Zhang K, Wan B, Sun X. CYP1B1 and MYOC mutations in 116 Chinese patients with primary congenital glaucoma. *Arch Ophthalmol* 2008;126(10):1443-1447.

19 Tanwar M, Dada T, Sihota R, Das TK, Yadav U, Dada R. Mutation spectrum of CYP1B1 in North Indian congenital glaucoma patients. *Mol Vis* 2009;15:1200-1209.

20 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;3:16021.

21 Ohtake Y, Tanino T, Suzuki Y, Miyata H, Taomoto M, Azuma N, Tanihara H, Araie M, Mashima Y. Phenotype of cytochrome P4501B1 gene (CYP1B1) mutations in Japanese patients with primary congenital glaucoma. *Br J Ophthalmol* 2003;87(3):302-304.

22 Kim HJ, Suh W, Park SC, Kim CY, Park KH, Kook MS, Kim YY, Kim CS, Park CK, Ki CS, Kee C. Mutation spectrum of CYP1B1 and MYOC genes in Korean patients with primary congenital glaucoma. *Mol Vis* 2011;17:2093-2101.

23 Chavarria-Soley G, Sticht H, Aklillu E, Ingelman-Sundberg M, Pasutto F, Reis A, Rautenstrauss B. Mutations in CYP1B1 cause primary congenital glaucoma by reduction of either activity or abundance of the enzyme. *Hum Mutat* 2008;29(9):1147-1153.

24 Su CC, Liu YF, Li SY, Yang JJ, Yen YC. Mutations in the CYP1B1 gene may contribute to juvenile-onset open-angle glaucoma. *Eye (Lond)* 2012;26(10):1369-1377.

25 Hollander DA, Sarfarazi M, Stoilov I, Wood IS, Fredrick DR, Alvarado JA. Genotype and phenotype correlations in congenital glaucoma: CYP1B1 mutations, goniodysgenesis, and clinical characteristics. *Am J Ophthalmol* 2006;142(6):993-1004.

26 Achary MS, Reddy AB, Chakrabarti S, Panicker SG, Mandal AK, Ahmed N, Balasubramanian D, Hasnain SE, Nagarajaram HA. Disease-causing mutations in proteins: structural analysis of the CYP1B1 mutations causing primary congenital glaucoma in humans. *Biophys J* 2006;91(12):4329-4339.

27 Choudhary D, Jansson I, Sarfarazi M, Schenkman JB. Characterization of the biochemical and structural phenotypes of four CYP1B1 mutations observed in individuals with primary congenital glaucoma. *Pharmacogenet Genomics* 2008;18(8):665-676.

28 Kaur K, Mandal AK, Chakrabarti S. Primary congenital glaucoma and the involvement of CYP1B1. *Middle East Afr J Ophthalmol* 2011;18(1):7-16.

29 Vasiliou V, Gonzalez FJ. Role of CYP1B1 in glaucoma. *Annu Rev Pharmacol Toxicol* 2008;48(1):333-358.

30 Prat C, Belville C, Comptour A, Marceau G, Clairefond G, Chiambaretta F, Sapin V, Blanchon L. Myocilin expression is regulated by retinoic acid in the trabecular meshwork-derived cellular environment. *Exp Eye Res* 2017;155:91-98.

31 Teixeira LB, Zhao Y, Dubielzig RR, Sorenson CM, Sheibani N. Ultrastructural abnormalities of the trabecular meshwork extracellular matrix in Cyp1b1-deficient mice. *Vet Pathol* 2015;52(2):397-403.

32 Williamson KA, FitzPatrick DR. The genetic architecture of microphthalmia, anophthalmia and coloboma. *Eur J Med Genet* 2014;57(8):369-380.

33 Banerjee A, Chakraborty S, Chakraborty A, Chakrabarti S, Ray K. Functional and structural analyses of CYP1B1 variants linked to congenital and adult-onset glaucoma to investigate the molecular basis of these diseases. *PLoS One* 2016;11(5):e0156252.

34 Zhao Y, Wang SJ, 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(21):4225-4240.

35 Tanwar M, Dada T, Sihota R, Dada R. Mitochondrial DNA analysis in primary congenital glaucoma. *Mol Vis* 2010;16:518-533.

36 Choudhary D, Jansson I, Schenkman JB. CYP1B1, a developmental gene with a potential role in glaucoma therapy. *Xenobiotica* 2009;39(8): 606-615.

37 Bolinches-Amorós A, Lukovic D, Castro AA, León M, Kamenarova K, Kaneva R, Jendelova P, Blanco-Kelly F, Ayuso C, Cortón M, Erceg S. Generation of a human iPSC line from a patient with congenital glaucoma caused by mutation in CYP1B1 gene. *Stem Cell Res* 2018;28:96-99.