CYP1B1 and *MYOC* Gene Analysis of Patients with Primary Congenital Glaucoma in the Cukurova Region of Türkiye

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Abstract

The aim of this study was to investigate the *CYP1B1* and *MYOC* genes in patients with primary congenital glaucoma (PCG) from the Cukurova region (located in the south of Türkiye) and reveal the relationship between gene mutations and clinical severity of the disease. Molecular genetic and clinical study was conducted in 42 eyes of 26 patients who were followed for a diagnosis of PCG. The clinical diagnosis was concluded by ophthalmological examination under general anesthesia or slit-lamp biomicroscopy, gonioscopy, and measurement of the intraocular pressure. A *CYP1B1* gene mutation was detected in 12 patients (46.2%). Two of these patients had a combination of CYP1B1 and MYOC mutations. The most common pathogenic variant, c.1405C > T (p.R469W) ($n = 5$), was present in patients with mutations, and the prognosis was poor compared with other modifications ($p = 0.014$). The second most common variant was c.3987G > A (p.G61E) ($n = 3$), which was associated with a good prognosis. The incidence of buphthalmos and the mean horizontal corneal diameter were higher in patients with mutations in the *CYP1B1* and *MYOC* genes. All parents were found to be carriers of the mutation gene. This is the report on molecular genetic analysis of PCG in the southern region of Türkiye. Some specific genetic variants may have an effect on the prognosis of the disease. However, patients without mutations in these case groups may have mutations in genes yet to be identified.

Keywords
- CYP1B1
- MYOC
- pathogenic variant
- primary congenital glaucoma

Introduction

Primary congenital glaucoma (PCG), which generally shows an autosomal recessive inheritance pattern and is a rare disease in our society, is the most common childhood glaucoma.[1] Aqueous humor drainage is disturbed owing to developmental anomalies in the anterior chamber angle and the trabecular meshwork, as well as an increase in the intraocular pressure (IOP) in the eye. This increase in IOP results in the enlargement of the globe and cornea (buphthalmos), corneal opacity, edema, Descemet membrane's rupture (Haab's striae), thinning of the sclera, deepening of the anterior chamber, and formation of atrophies in the iris. Further, it impairs the feeding of the optic disk. Increased IOP can result in blindness and vision loss and blindness if the patient is untreated or undertreated.[2] PCG is the most common type of glaucoma in childhood, with a global incidence rate of approximately 110,000 births.[3] This disease

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exhibits a high prevalence in populations where consanguinity is common, such as in Saudi Arabia (1:2,500) and among a population in southern India (1:3,300).[4,5] Five chromosomal loci are currently associated with the disease: GLC3A (chromosome 2p21), GLC3B (chromosome 1p36.2-p36.1), GLC3C (chromosome 14q24.3), GLC3D (chromosome 14q24.2-q24.3), and GLC3E (chromosome 9p21.2).[6] Nevertheless, only three genes were found to be involved in the development of PCG: CYP1B1 (cytochrome P450, family 1, subfamily B, polypeptide 1), located in the GLC3A locus; LTBP2 (latent-transforming growth factor beta-binding protein 2), located in the GLC3D; and TEK (tyrosine kinase receptor), located in the GLC3E. The role of the proteins encoded by these genes in the etiology of the disease remains unclear.[6,7] Mutations in PITX2 (pairedlike homeodomain transcription factor 2) and FOXC1 (forkhead box C1) are associated with Axenfeld–Rieger syndrome (ARS). In addition, the paired box six gene mutation is associated with aniridia. Moreover, these three gene mutations are observed in PCG. Furthermore, the association of MYOC (myocilin) gene mutation with PCG, which is associated with primary open-angle glaucoma in adults, has been reported.[8] This study aimed to investigate the genetic basis of PCG in the Cukurova region (located in southern Türkiye) and to reveal the relationship between the detected gene mutations and the clinical findings and disease severity. Revealing the gene mutations that cause the condition can guide the genetic counseling services to these patients including their families, and informing the couples at risk. Further, associating the responsible mutations in PCG cases with various clinical forms may guide clinical follow-up and prediction of prognosis of these cases.

Materials and Methods

Clinical Evaluation and Patient Selection

Peripheral blood samples were sent to the Cukurova University Department of Medical Genetics & Adana Genetic Disease Diagnosis and Treatment Center (AGENTEM) referred for molecular genetic testing and included in this study. Peripheral blood samples were collected for genomic deoxyribonucleic acid (DNA) isolation using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The quality of the DNA samples was assessed with a Qubit fluorimeter (Thermo Fisher Scientific, Waltham, Massachusetts, United States). Informed parental consent was obtained for all patients following the ethical standards of the institutional ethics committee (Cukurova University Faculty of Medicine Noninvasive Clinical Research Ethics Commission) and the Declaration of Helsinki. The diagnosis of PCG was made based on the following criteria: (1) age of onset 3 years; (2) corneal diameter (at least 10 mm) and presence of Haab's striae, corneal edema, or both; (3) IOP ($\geq$21 mm); and (4) optical disk cupping (cup-to-disk [C/D] ratio) C 0.3 or more than the difference of 0.2 between both C/D ratios.[9] We excluded patients with secondary glaucoma owing to systemic and ocular anomalies (cataracts and corneal dystrophy) or patients with juvenile glaucoma from the study. Patients who were diagnosed with PCG and without advanced glaucoma damage underwent medical treatment. Patients with advanced glaucoma damage underwent trabeculotomy, trabeculectomy, or trabeculectomy with mitomycin C. IOP was measured at the beginning of anesthesia (usually under halothane anesthesia) using a TonoPen. Those who showed compliance with the slit-lamp examination were evaluated using Goldmann's applanation tonometry. Corneal diameters were measured with the Castroviejo caliper. The clinical results of individuals with *CYP1B1* and *MYOC* gene variations were compared with those without a detected variant to investigate the association between genotype and phenotype. Thus, several clinical parameters were assessed, such as age at diagnosis, age at evaluation, IOP at evaluation, horizontal corneal diameter, bilateral disease, corneal thickness, surgical interventions, antiglaucoma medications, corneal haze, Haab's striae, buphthalmos, C/D ratio, blindness, and consanguinity.

Analysis of the *CYP1B1* and *MYOC* Gene

Next-generation sequencing: The next-generation sequencing workflow was performed to achieve a minimum of 300x coverage on an Illumina MiSeq (San Diego, California, United States) platform via a custom-designed panel, including the *CYP1B1* and *MYOC* genes, by AGENTEM. This panel included all exons, introns, and exon–intron junctions.

Bioinformatics analyses: Quality control parameters for sequencing and variant qualities were checked via the QCI Analyze tool and the QCI Interpret interface. Total yield, sequencing quality score, depth of coverage, a quality score of variants, forward/reverse read balance, population, and variant frequencies were assessed as primary variant analyses. In addition, variants were categorized based on their pathogenicity according to the American College of Medical Genetics (ACMG) criteria as pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign, or benign. In silico analysis tools, including SIFT, B-SIFT, PolyPhen-2, MutationTaster, BLOSUM, PROVEAN, CADD, DANN, GeneSplicer, PhyloP, MaxEntScan, and QCI Inferred Activation, were also used for the further examination of the VUSs.

Statistical Analysis

A Microsoft Excel spreadsheet was created to analyze the data, which was then transferred into SPSS software (version 20.0; IBM Corp., Chicago, Illinois, United States). Mann–Whitney *U* test was performed to compare the means of the two independent samples, and Fisher's exact test was performed to compare categorical variables. In all analyses, $p < 0.05$ was considered statistically significant.

Results

42 eyes of 26 patients were included in the study, of which 12 (46.2%) were boys and 14 (53.8%) were girls. Genetic analysis of the 26 patients with PCG revealed the presence of a *CYP1B1* gene mutation in 12 (46.2%) patients and *MYOC* gene mutation combination in two (7.7%) patients. While there was no

Table 1 Clinical parameters and demographic profile of 26 patients ($n = 42$ eyes) with and without mutations of the *CYP1B1* and *MYOC* gene

	Patients with mutations ($n = 12$)	Patients without mutations ($n = 14$)	Total	*p*
IOP (mm Hg), mean ± SD	30.65 ± 10.29	29.27 ± 13.03	29.92 ± 11.68	0.70
HCD (mm), mean ± SD	13.25 ± 0.19	11.38 ± 1.69	12.26 ± 2.03	0.006
AXL (mm), mean ± SD	22.75 ± 3.27	21.23 ± 2.98	21.84 ± 3.13	0.20
Corneal edema	10 (50%)	12 (54.5%)	22 (52.4%)	0.45
CCT (μm), (mean ± SD)	614.6 ± 72.2	600.2 ± 83.6	607.7 ± 766	0.64
Buphthalmos	11 (55%)	6 (35.3%)	17 (40.5%)	0.057
Cup/disc ratio	0.49 ± 0.47	0.48 ± 0.42	0.48 ± 0.43	0.97
Topical treatment	16 (80%)	17 (77.3%)	33 (78.6%)	0.22
Surgical treatment	15 (75%)	20 (90.9%)	35 (83.3%)	0.63

Abbreviations: AXL, axial length; CCT, central corneal thickness; HCD, horizontal corneal diameter; IOP, intraocular pressure; PCG, primary congenital glaucoma.
Note: Statistical analysis was performed with generalized estimating equating method.
$p < 0.05$ = statistical significance.

difference in bilateral eye involvement, IOP values, axial length, corneal thickness, or corneal edema in patients with mutation compared with patients who did not, it was evident that buphthalmos was more common. In addition, the horizontal corneal diameter was larger (►**Table 1**). The mutation site of 12 patients with the *CYP1B1* gene mutation was the second and third exons. Seven (46.7%) of these mutation sites were on the second exon and eight (53.4%) were on the third exon. The pathogenic variant c.1405C > A (p.R469W) was observed in five patients with the *CYP1B1* gene mutation in the third exon site, of which four (75%) of these mutations were homozygous and one (25%) was heterozygous. The second most common mutation c.182G > A (p.G61E) with CYP1B1 was observed in three patients with the *CYP1B1* gene mutation at the second exon site. Nine different mutations were seen in *CYP1B1* and *MYOC* genes (►**Table 2**). All parents were found to be carriers of the gene. We also investigated the relationship between clinical prognosis and genetic variants for patients with or without *CYP1B1* gene mutations. Poor prognosis criteria are high IOP values despite medical and surgical treatment, need for additional surgical treatment (revision surgeries, seton surgery, keratoplasty, lensectomy, vitrectomy), increased optic cupping rate, persistent corneal edema, and corneal scarring or phthisis bulbs. Pathogenic variant analysis was examined and it was observed that all patients with the most common c.1405C > A (p.R469W) variants of the *CYP1B1* gene had a poor prognosis. However, it was observed that the prognosis was good in patients with the c.182G > A (p.G61E) *CYP1B1* gene variant, which is the second most common pathogenic variant ($p = 0.014$; ►**Fig. 1**).

Discussion

In this study, *CYP1B1* gene mutations were detected in 46.2% of patients with PCG. The most common pathogenic variant was c.1405 C > T (p.R469W), and c.182G > A (p.G61E) was detected in the second line. *CYP1B1*, a gene that causes PCG, was discovered to be inherited in an autosomal recessive fashion and *MYOC* was inherited in an autosomal dominant fashion.[10] In the genetic analysis of two patients, co-occurrence of mutations in the *CYP1B1* and *MYOC* genes was shown. Few studies have reported that PCG is observed in both boys and girls; similar rates were observed in both genders in our study, 14 (53.8%) females and 12 (46.2%) males.[11,12] The *CYP1B1* gene contains three exons with lengths of 371, 1,044, and 3,707 bp. This gene has two introns that contain 8.5 kb of genomic DNA. Coding generally starts from the second exon and ends in the third exon. The first exon is 371 bp long. It is coded with 16% of the second and third exons.[13] The most frequent mutation sites in the *CYP1B1* gene mutation were the second and third exons.[14] In our study, 46.7% of the mutations were found in the second exon and 53.3% in the third exon. This rate is consistent with the study that reported 45% mutations in the second exon.[15] *CYP1B1* produces the cytochromp4501b1 protein, which contains 543 amino acids. It is a submember of the cytochrome p450 enzyme. This enzyme is localized in the endoplasmic reticulum. It metabolizes procarcinogens, such as polycyclic aromatic hydrocarbons and 17-beta-estradiol.[16] This enzyme also metabolizes a steroid that promotes eye development. The aqueous humor produced in the ciliary body causes IOP. With the aid of the trabecular meshwork, the aqueous humor enters the systemic circulation through the eye. The *CYP1B1* gene was detected in these two eye tissues. Some studies have reported that the *CYP1B1* gene is required to develop the trabecular meshwork.[16,17] The effect of the mutation in this gene on the development of the trabecular meshwork and other ocular tissues has yet to be fully explained. However, these mutations are believed to reduce the enzyme's catalytic activity, stability, and amount of enzyme. R469W is in the "heme-binding" region of the cytochrome p450 enzyme. This mutation disrupts the normal function of hemoproteins. The stability of the hemoprotein complex decreases with G61E and increases with

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Table 2 Clinical characteristics of patients with a genetic mutation in the *CYP1B1*(4,6. Case *MYOC* combination) gene

Case	Location	Nucleotide change, pathogenic variant	Sex	Mutation type	Mode of inheritance	Age (mo)	Affected eye	IOP mean (mm Hg)	Corneal diameter (mm) OD/OS	C/D OD/OS	Clinical sign	Prognosis
1	Exon 2,3	c.1405 C > T (p.R469W)	M	Heterozygous	AR	3.5	Bilateral	42/30	14.5/14.5	0.4/0.4	Bilateral buphthalmus	Poor
2	Exon 3	c.1405 C > T (p.R469W)	M	Homozygous	AR	14	Bilateral	28/34	15/15	0.8/1.0		Poor
3	Exon 3	c.1405 C > T (p.R469W)	F	Homozygous	AR	4	Bilateral	32/34	12/12	0.8/0.7		Poor
4	Exon 3 Exon 2 (MYOC)	c.1405 C > T (p.R469W) c.634T > G (p.F212V)	M	Homozygous Heterozygous	AR	4	Bilateral	27/27	14/13	0.9	Bilateral buphthalmus	Poor
5	Exon 2,3	c.1405 C > T (p.R469W)	F	Homozygous	AR	6	OS	29	12	0.3	Buphthalmus	Poor
6	Exon 2 Exon 2 (MYOC)	c.182G > A (p.G61E) c.366C > A (p.G112G)	M	Heterozygous Heterozygous	AR	2	OD	28	11	0.8		Good
7	Exon 2	c.947A > T (p.D316V)	F	Heterozygous	AR	7	OD	21	12	0.3	Buphthalmus	Good
8	Exon 2	c.182G > A (p.G61E)	F	Homozygous	AR	1	OD	34	11	0.5		Good
9	Exon 3	c.947A > T (p.D316V)	F	Heterozygous	AR	1	Bilateral	48/48	12/11	0.6/0.5		Poor
10	Exon 2,3	c.1200_1209 dup10bp (p.T404Sfs*30) c.182G > A (p.G61E)	M	Heterozygous Heterozygous	AR	1	Bilateral	24/25	11/12	0.5/0.5		Good
11	Exon 2	c.868dupC (p.R290P)	M	Homozygous	AR	4	Bilateral	47/39	16/16	1.0/0.9	Bilateral buphthalmus	Poor
12	Exon 2	c.868dupC (p.R290P)	F	Homozygous	AR	4	Bilateral	21/34	15/16	0.6/0.7	Bilateral buphthalmus	Poor

Abbreviations: AR, autosomal recessive; C/D, cup-to-disk ratio; IOP, intraocular pressure; OD, oculus dexter; OS, oculus sinister.

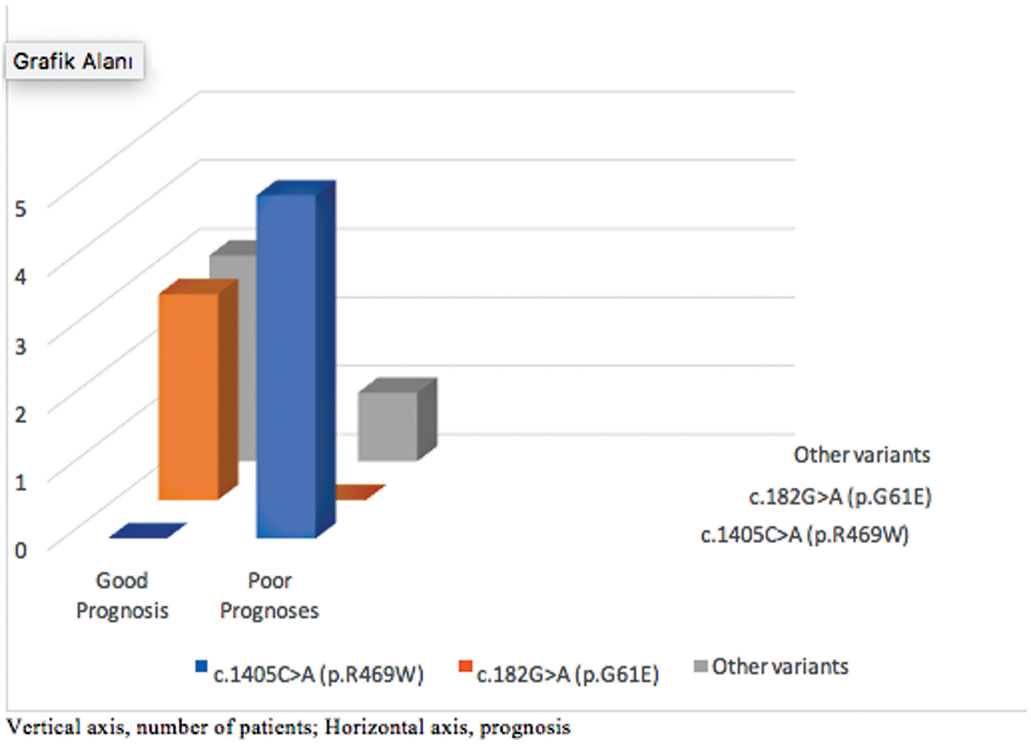

Fig. 1 *CYP1B1* gene pathogenic variants prognosis association ($n = 12$).

R469W. The decrease in hydroxylation of 17α-estradiol was higher in c.182G > A (p.G61E) than in c.1405 C > T (p.R469W) mutations.[18] *CYP1B1* is a tumor biomarker because of its overexpression in tumor cells derived from among others: breast, colon, lung skin, brain, and testis. Stilbene, flavonoid, coumarin, and anthraquinone are the four major types of compounds that inhibit *CYP1B1* activity. *CYP1B1* expression may suggest a therapeutic benefit for multiple diseases such as glaucoma and cancer. The design of efficient and safe *CYP1B1* inhibitors is still a developing area of research.[19] The most common cause of PCG is a mutation in the *CYP1B1* gene, and 150 variants have been defined throughout the world.[20] Studies have shown that the *CYP1B1* gene mutation shows different distributions between different populations worldwide. In countries such as those in the Middle East, Saudi Arabia, and Slovakia, 70 to 100% of cases of PCG are due to the *CYP1B1* gene mutation. According to studies, this percentage is 50% in Brazil and France, 20% in Japan, 14 and 17% in Ecuador and China, respectively, and 10% in Mexico.[14,21] The rate of our study was 46.2%. In a comprehensive meta-analysis study, 147 different mutations in the *CYP1B1* gene were observed in 542 patients. Among these mutations, the most common c.3987G > A (p.G61E) mutation (18.85%) was found, and 88% of the patients with this mutation were from countries in the Middle East. The second most common mutation was c.7940G > A/T (p.R368H/L), found mainly among Caucasians (68.54%).[15] Studies conducted in Türkiye report that the rate of c.182G > A (p.G61E) mutations is 23%.[22] It was found to be 11.53% in our study. At the same time, unlike other studies, the c.1405 C > T (p.R469W) mutation occurred in 19.23% of cases. In our study, there was no difference between the groups with and without mutation in bilateral eye involvement, axial length, corneal thickness, and optic cupping rate. Similarly, in other studies, no correlation was found between the severity of the glaucoma phenotype and the *CYP1B1* gene mutation.[23] However, unlike other studies, buphthalmos were more common and the horizontal diameter of the cornea was larger in patients with mutations. Many factors contribute to corneal edema in patients with PCG. At the same time, the *CYP1B1* genetic mutation is associated with central corneal edema.[24–26] However, a rate of 52.4% of participants in our research experienced corneal edema, which was not associated with the existence of the mutation. Taking into account the relationship between treatment and prognosis, it was observed that prognosis was good in most patients after medical and surgical treatment in patients without mutations. On the contrary, patients with mutations had a worse prognosis despite medical and surgical treatment. When pathogenic variants of patients with the mutation were examined in our study, the prognosis was poor in all patients with c.1405 C > T (p.R469W) mutation despite treatment, and the prognosis was good in all patients with c.182G > A (p.G61E) mutation under treatment. These genetic analyses provide essential data regarding the prediction and early diagnosis of the disease. The limitations of our study include the retrospective nature of clinical data, and the limited number of cases included. Additionally, *FOXC1* and *PITX2*, reported in previous studies, are other mutations that were not evaluated in our research. As a result, revealing the gene mutations that cause the disease can guide genetic counseling services to these patients and their families, and

informing couples at risk may have an effect on outcomes. In addition, however, associating the responsible mutations in patients with PCG with various clinical forms may guide clinical follow-up and prognosis estimation of these cases.

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Conflict of Interest
None declared.

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