• Clinical Research •

Novel CYP1B1 mutations and a possible prognostic use for surgical management of congenital glaucoma

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Received: 2018-07-08 Accepted: 2018-11-09

Abstract

• AIM: To identify CYP1B1 gene mutations and evaluate their possible role as a prognostic factor for success rates in the surgical management of Egyptian congenital glaucoma patients.

• METHODS: Totally 42 eyes of 29 primary congenital glaucoma patients were operated on with combined trabeculotomy/trabeculectomy with mitomycin-C and followed up at 1d, 1wk, 1, 6 and 12mo postoperatively. Genomic DNA was extracted from peripheral blood leukocytes. Coding regions of CYP1B1 gene were amplified using 13 pairs of primers, screened for mutations using single-strand conformation polymorphism followed by sequencing of both strands. Efficacy of the operation was graded as either a success [maintaining intraocular pressure (IOP) less than 21 mm Hg with or without anti-glaucoma medication], or a failure (IOP more than 21 mm Hg with topical antiglaucoma medications).

• RESULTS: Seven novel mutations out of a total of 15 different mutations were found in the CYP1B1 genes of 14 patients (48.2%). The presence of CYP1B1 gene mutations did not correlate with the failure of the surgery (*P*=0.156, odds ratio=3.611, 95%CI, 0.56 to 22.89); while the positive consanguinity strongly correlated with failure of the initial procedure (*P*=0.016, odds ratio=11.25, 95%CI, 1.57 to 80.30). However, the Kaplan-Meier survival analysis revealed a significantly lower time of IOP control in the subgroup with mutations in CYP1B1 versus the congenital primary glaucoma group without mutations (log rank test, *P*=0.015).

• CONCLUSION: Seven new CYP1B1 mutations are identified in Egyptian patients. Patients harboring confirmed mutations suffered from early failure of the initial surgery. CYP1B1 mutations could be considered as a prognostic factor for surgery in primary congenital glaucoma.

• KEYWORDS: CYP1B1 mutations; primary congenital glaucoma; intraocular pressure; trabeculotomy/trabeculectomy with mitomycin-C.

DOI:10.18240/ijo.2019.04.14

Citation: Khafagy M, El-Guendy N, Tantawy M, Eldaly M, Elhilali H, Abdel Wahab A. Novel CYP1B1 mutations and a possible prognostic use for surgical management of congenital glaucoma. *Int J Ophthalmol* 2019;12(4):607-614

INTRODUCTION

Primary congenital glaucoma (PCG) is the most common childhood glaucoma accounting for 50%-70% of the cases[1-3]. It occurs due to the abnormal development of the trabecular meshwork (TM) and the anterior chamber angle. This anomaly is called trabeculodysgenesis or iridotrabeculodysgenesis according to the tissues involved[1,4]. It is characterized by an elevation in intraocular pressure (IOP) in the early infantile period, which causes structural damage to the eyeball such as buphthalmos, corneal edema, Haab's striae and may result in optic nerve damage and subsequent permanent loss of vision[3,5]. The combined trabeculotomy-trabeculectomy procedure with mitomycin-C (MMC) was recommended as a primary procedure in moderate to severe cases[6-7].

About 10%-40% of PCG cases are familial and may be associated with consanguinity[1,8]. Its incidence varies geographically; PCG is four times more common in Saudi Arabia than in Western societies with an incidence of 1:2500 and 1:10 000 live births respectively[1,3,8]. Four different loci have been linked to PCG (GLC3A, GLC3B, GLC3C and GLC3D)[8-12]. Mutations in the cytochrome P4501B1 gene (CYP1B1) located in the GLC3A locus on chromosome 2p21 are the most predominant (Online Mendelian Inheritance in Man, OMIM, ID No.231300). They were first reported in PCG patients in 1997[13].

Sequence analyses have so far revealed approximately 243 different mutations in the CYP1B1 gene (Human Genome Mutation database; http://www.hgmd.cf.ac.uk/ac/index.php), of which about 159 are implicated in the pathogenesis of PCG which indicates excessive allelic heterogeneity[11,14-15]. The mutation spectra of CYP1B1 varies widely across different populations[15]; from almost 100% among Saudi Arabians[16] and Slovakian Gypsies[17], to 50% among Brazilians, and 20% in Japanese[13,18-19]. It was found that patients with CYP1B1 gene mutations required more surgeries to control the IOP than patients without these mutations[20-21].

Very little investigation has been done to study CYP1B1 and PCG in Egyptian patients. In this work, we perform a preliminary investigation to identify CYP1B1 mutations in PCG Egyptian patients and to evaluate the role of CYP1B1 mutations as a prognostic factor predicting the outcome of surgery in a cohort group of Egyptian PCG patients operated on with combined trabeculotomy-trabeculectomy with MMC.

SUBJECTS AND METHODS

Ethical Approval This prospective interventional study was approved by the Research Ethics Committee of Cairo University Hospitals and the declaration of Helsinki was respected. Subjects for the study were recruited randomly from the patients attending the Cairo University Pediatric Ophthalmology clinic who met the inclusion criteria for the study. All parents of patients signed an informed consent for the surgery and inclusion in the study.

Patients Inclusion criteria entailed being an Egyptian primary congenital glaucoma patient who was not operated on before in at least one eye and age of onset ranging from 0-2y. Secondary glaucoma patients and patients having additional developmental ocular and/or systemic anomalies were excluded from the study.

Surgical Procedure The patients were subjected to full ophthalmic assessment to confirm meeting the inclusion criteria, including detailed anterior segment examination with measurement of the horizontal corneal diameter, measurement of the IOP using Perkins applanation tonometer under general anesthesia, posterior segment evaluation (by ophthalmoscopy or ultrasonography), and axial length measurement. The clinical severity of the cases was assessed using a severity scale of mild, moderate and severe as proposed earlier[6]. Children meeting the inclusion criteria underwent combined trabeculectomy-trabeculotomy with MMC done by the same surgeon in all patients. Postoperatively, the patients underwent full ophthalmic assessment (under sedation with oral chloral hydrate 25 mg/kg, once, 30min prior to examination) at one day, one week, and then monthly for one year including measurement of IOP, horizontal corneal diameter, and optic nerve head evaluation. Surgical result of the initial operation was graded into either success (maintaining IOP less than 21 mm Hg with or without anti-glaucoma medication at the end of the follow-up period), or failure (IOP more than 21 mm Hg with topical antiglaucoma medications at any point during the follow-up period).

CYP1B1 Gene Mutation Detection Peripheral blood samples were collected, at the time of the initial procedure, and genomic DNA was extracted using Generation capture column kit (Gentra, USA) following the manufacturer's protocol. The coding regions in CYP1B1 were amplified using 13 pairs of primers from a previous study[22].

Polymerase chain reaction (PCR) amplification of the coding regions of the CYP1B1 gene from genomic DNA and screening with single-strand conformation polymorphism (SSCP) were performed as previously described[22]. PCR products with SSCP variants were purified using Axyprep PCR cleanup kits (Axygene Biosciences, USA) adhering to the manufacturer's protocol. Analysis of the sequencing reactions was performed using Big Dye Terminator cycle sequencing kit (Applied Biosystem, Foster City, CA) on ABI Prism 3730 Genetic Analyzer automated sequencer (Applied Biosystems, Foster City, CA). For confirmation, sequencing was done in forward and reverse strands in case of suspected sequence alteration.

The sequence alignment was done against the wild-type CYP1B1 sequence (Genbank U56438.1) using the LALIGN software server (http://www.ch.embnet.org/software/LALIGN_form.html). CodonCode aligner software (CodonCode Corp., Centerville MA, USA) was used for multiple alignment and comparison of different sequences. The amino acid sequence for the CYP1B1 was obtained from The National Center for Biotechnology Information (NCBI) Reference Sequence: NP_000095.2, and sequence alterations were studied using DNASIS® Max v3.0 software (Hitachi Solutions America, Ltd., San Francisco, USA). Evolutionary conserved regions were studied by comparing amino acid sequences across different species (obtained from NCBI). The impact of missense mutations was predicted by using the mutation-tolerance-prediction software (SIFT) provided by Genome Institute of Singapore, available on the website (http://sift.bii.astar.edu.sg/). Patients with confirmed CYP1B1 sequence alteration were designated "mutation-present", while those who could not be confirmed with sequence alteration were designated "mutation-absent".

Statistical Analysis All statistical analyses were done using IBM SPSS v20.0 software (IBM Corporation, USA). Descriptive statistics were calculated, and the data were summarized as mean±standard deviation (SD) for continuous variables. Percentages and tables were used to describe categorical data.

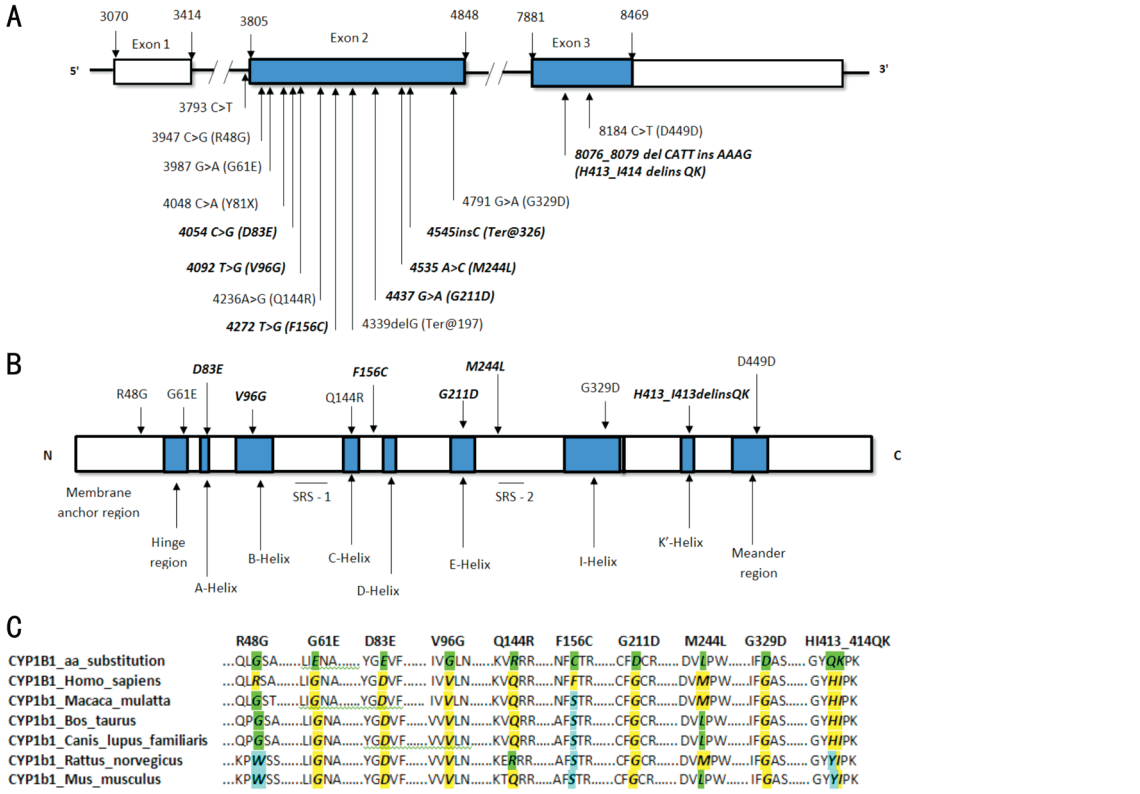

Figure 1 CYP1B1 genomic, protein structure and homology A: CYP1B1 genomic structure with intron and exon boundaries locations (coding exons are shaded in blue) and positions of mutations and SNPs identified in the study. Novel mutations are shown in bold & italics. Nucleotide positions are in reference to CYP1B1 GenBank accession number U56438. B: CYP1B1 protein diagram showing locations of detected mutation marked on different regions of the protein. The evolutionary conserved regions are shaded in blue with annotations below. Locations of different missense mutations detected in the study are outlined above. Novel mutations are shown in bold. Short horizontal lines indicate substrate recognition sites (SRSs). C: Comparison of CYP1B1 protein sequence among species to detect evolutionary conservation at the areas of missense mutations.

Comparisons between categorical data were done using Pearson's Chi-square test or Fisher's exact test. Comparisons of non-paired continuous variables were done using independent samples *t*-test, while comparisons of paired continuous variables were carried out using paired sample *t*-test. Correlations between non-parametric variables were done by Spearman's rank correlation coefficient. Cumulative success of the initial procedure between both groups was examined using Kaplan-Meier survival analysis. A *P*-value <0.05 was considered statistically significant.

RESULTS

Patients Data The study included 42 eyes in 29 Egyptian children who underwent combined trabeculotomy-trabeculectomy with MMC as an initial procedure and were followed-up for 12mo postoperatively. Table 1 summarizes the demographic data collected from the 42 eyes (29 patients) included in the study. The mean age at presentation of the 29 patients was 8.57±10.63mo. No difference was found in clinical severity between the two subgroups in our study (Table 2). Table 3 shows the summary of the preoperative and postoperative IOP and corneal diameter of the 42 eyes included in the study.

Table 1 Demographic data

Parameters	Patients	Eyes
Gender (male/female)	18/11	24/18
Family history (positive/negative)	4/25	6/36
Consanguinity (positive/negative)	9/20	15/27

Table 2 Preoperative clinical data of the eyes included in the study in both subgroups

Parameters	Mutation-detected	Mutation-not-detected	P^a
Initial IOP	26.6±3.5	27.9±4.6	0.289
Initial corneal diameter	13.4±0.7	13.1±0.6	0.077
Initial axial length	22.1±1.8	22.2±1.6	0.943

IOP: Intraocular pressure. [a]Independent samples *t*-test.

Mutation Detection All PCR products were subjected to SSCP to detect possible mutations through variation in band migration. Most band shifts appeared in the fragments corresponding to exon 2 where the Hinge region and both substrate binding sites are located (Figures 1A). DNA sequencing of the shifted fragments identified 15 different mutations, in 14 patients, that mostly lie in the second exon (Figures 1B, Table 4). Eight of these alterations were previously reported in different populations (HGMD; http://

Table 3 Preoperative and postoperative IOP and corneal diameter from eyes included in the study mean±SD

Parameters	Preoperative	Postoperative					
		1d	1wk	1mo	3mo	6mo	12mo
IOP (mm Hg)	27.30±4.11	12.19±3.40	14.02±3.86	15.97±5.34	16.90±6.12	17.85±6.32	18.5±7.42
P^a	N/A	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Corneal diameter (mm)	13.23±0.69	13.23±0.69	13.22±0.67	13.20±0.65	13.17±0.65	13.17±0.65	13.17±0.65
P^a	N/A	N/A	0.323	0.183	0.058	0.058	0.058

IOP: Intraocular pressure; N/A: Not available. [a]Paired samples *t*-test *vs* preoperative.

Table 4 Summary of CYP1B1 mutations/SNP observed in 29 Egyptian PCG patients

No.	Sequence alteration[a]	Frequency	Protein	Location	Damage[b]	Status	Type	Reported[c]/novel
1	3793 C>T (IVS1-12 C>T)	2	No change	Intron 1	No	T/T, T/C (Homozygous, Heterozygous)	SNP	Stoilov *et al*[23], 1998 (rs2617266)
2	3947 C>G	4	R48G	Exon 2, Hinge region	No	G/G (Homozygous)	SNP	Stoilov *et al*[23], 1998 (rs10012)
3	3987 G>A	5	G61E	Exon 2, Hinge region	Yes	A/A (Homozygous)	Mutation	Bejjani *et al*[16], 1998
4	4048 C>A	1	Y81X	Exon 2, A-Helix	Yes	A/A (Homozygous)	Mutation	Chitsazian *et al*[24], 2007
5	4054 C>G	2	D83E	Exon 2, A-Helix	±	G/G (Homozygous)	Mutation	Novel
6	4092 T>G	1	V96G	Exon 2, B-Helix	Yes	T/G (Heterozygous)	Mutation	Novel
7	4236 A>G	1	Q144R	Exon 2, C-Helix	Yes	A/G (Heterozygous)	Mutation	Chakrabarti *et al*[25], 2003
8	4272 T>G	1	F156C	Exon 2	Yes	T/G (Heterozygous)	Mutation	Novel
9	4339delG	1	Frameshift (Ter@196)	Exon 2	Yes	Homozygous	Mutation	Belmouden *et al*[26], 2002
10	4437 G>A	1	G211D	Exon 2, E-Helix	Yes	A/A (Homozygous)	Mutation	Novel
11	4535 A>C	1	M244L	Exon 2	±	A/C (Heterozygous)	Mutation	Novel
12	4545insC	1	Frameshift (Ter@326)	Exon2	Yes	Homozygous	Mutation	Novel
13	4791 G>A	1	G329D	Exon 2, I Helix	Yes	A/A (Homozygous)	Mutation	Dimasi *et al*[27], 2007
14	8076-8079 del CATTinsAAAG	2	H413_I414 delins QK	Exon 3, K'-Helix	Yes	Homozygous	Mutation	Novel
15	8184 C>T	2	D449D	Exon 3, Meander	No	C/C (Homozygous)	SNP	Stoilov *et al*[23], 1998 (rs1056837)

SNP: Single nucleotide polymorphism. [a]Mutations are described in accordance with human CYP1B1 reference sequence at Genbank accession number U56438; [b]According to position on protein and tolerance simulation on SIFT result; [c]According to Human Genome Mutation Database (HGMD).

www.hgmd.cf.ac.uk/); three were previously reported SNP (3793 C>T, R48G, and D449D). Seven were novel mutations of which only 2 were predicted to be tolerated [4054 C>G (D83E) and 4535 A>C (M244L)], and the remaining 5 were suggested to be damaging mutation according to "Tolerance Simulation on SIFT". The novel mutation 4545insC resulted in terminating the protein at amino acid 326 eliminating exon 3 from the resulting truncated protein. All patients with novel mutations had more than one mutation except for patient 14 who had only 4545insC (Ter@326) chain termination mutation. The mutation most frequently found in this study is the previously reported damaging mutation G61E which was found in 5 patients. Most of the mutated amino acids lie in areas that are evolutionary conversed as shown in Figure 1C.

The genotype-phenotypes encountered in our study are summarized in Tables 5 and 6. Table 5 shows the 14 patients identified with different mutations. A wide variability in phenotype is noticed with all mutations. From Table 6, it is clear that mutations with severe phenotype and bad outcome were G61E, the truncating mutation Y81X and the frameshift 4339delG. One patient was homozygous while the other had compound heterozygous genotype (G61E+G211D). All these mutations were reported previously with severe phenotypes and bad prognosis[19].

Thirty-one eyes (76%) in 22 patients showed success, while eleven eyes (24%) in 7 patients showed failure of the initial procedure and required re-surgery. Five out of 7 patient with failed initial procedure had confirmed mutations. While 9 out of 22 patients with successful initial procedure had mutations (Table 7). Positive consanguinity was found to be strongly correlated to failure of the initial procedure (P=0.016) with an odds ratio of 11.25 (Table 7). Age at presentation was found to have a significant directly proportional correlation with survival time, *i.e.* the older the child, the longer the initial procedure controls the IOP (rho=0.542, P<0.001). A weaker inverse relationship (rho=-0.332, P=0.031) was present between initial IOP and the survival time (Table 8). Kaplan-Meier survival analysis was done to compare the survival time between the mutation-present and the mutation-absent subgroups a difference was revealed in favor of mutation-absent subgroup (Figure 2). This difference was found to be highly significant (log-rank test, P=0.015).

Table 5 Genotype-Phenotype correlations of the patients included in the study

No.	Detected mutation(s)	Type	Clinical severity[a]	Outcome of initial procedure
1	3793 C>T+R48G+V96G+G329D	Compound heterozygous	Moderate	Success
2	Q144R+F156C+8076-8079 del CATTinsAAAG	Compound heterozygous	Moderate	Success
3	R48G	Homozygous	Severe	Success
4	G61E+D83E+M244L	Compound heterozygous	Moderate	Success
5	4545insC (Ter@326)	Homozygous	Moderate	Success
6	3793 C>T+R48G+8076-8079 del CATTinsAAAG	Compound heterozygous	Moderate	Success
7	4339delG (Ter@196)	Homozygous	Severe	Failure
8	G61E+G211D	Compound heterozygous	Severe	Failure
9	R48G	Homozygous	Moderate	Success
10	G61E	Homozygous	Moderate	Success
11	G61E	Homozygous	Severe	Failure
12	G61E+D449D	Compound heterozygous	Severe	Failure
13	D449D	Homozygous	Moderate	Success
14	Y81X+D83E	Compound heterozygous	Severe	Failure

[a]Based on Al-Hazmi *et al*[6], 2005.

Table 6 Severe phenotype associated with mutations detected in Egyptian PCG patients

Detected mutation(s)	No. of patients having the mutation	Severe phenotype/total number of patients	Surgical failure/total patients
3793 C>T (IVS1 -12 C>T)	2	None	None
R48G	4	1/4 (25%)	None
G61E	5	3/5 (60%)	3/5 (60%)
Y81X	1	1/1 (100%)	1/1 (100%)
D83E	2	1/2 (50%)	1/2 (50%)
V96G	1	None	None
Q144R	1	None	None
F156C	1	None	None
Ter@196	1	1/1 (100%)	1/1 (100%)
G211D	1	1/1 (100%)	1/1 (100%)
M244L	1	None	None
Ter@326	1	None	1/1 (100%)
G329D	1	None	None
H413_I414 delins QK	2	None	None
D449D	2	1/2 (50%)	None

Table 7 Mutation status and severity in relation to outcome of the initial procedure

Mutation status	Present	Absent	*P*
Failure	5	2	0.159[a] (odds ratio=3.611, 95%CI, 0.56-22.89, *P*=0.173)
Success	9	13	
Total	14	15	
Consanguinity	Positive	Negative	
Failure	5	2	0.016[b] (odds ratio=11.25, 95%CI, 1.57-80.30, *P*=0.0158)
Success	4	18	
Total	9	20	

[a]Pearson's Chi-square=6.77; [b]Fisher's exact test.

Table 8 Correlations between different preoperative factors and the survival time of the initial procedure

Parameters	Spearman's rho	*P*
Age at presentation	0.542	<0.001
Presenting IOP	-0.332	0.031
Presenting corneal diameter	0.106	0.504
Presenting axial length	0.259	0.098

DISCUSSION

PCG is the most common childhood glaucoma. Despite being rare, its occurrence increases 10 times in some parts of the world because of consanguinity[28]. It has been repeatedly found that mutations in CYP1B1 are involved in the development of the disease. Mutations of this gene have not been properly analyzed in Egypt. In this study, we presented the association between mutations of the CYP1B1 gene and the failure of surgery in PCG. It was found that patients with confirmed CYP1B1 mutations were more likely to fail their initial surgical procedure earlier than patients without mutations as shown from the survival analysis of the surgeries done to the patients enrolled in the study.

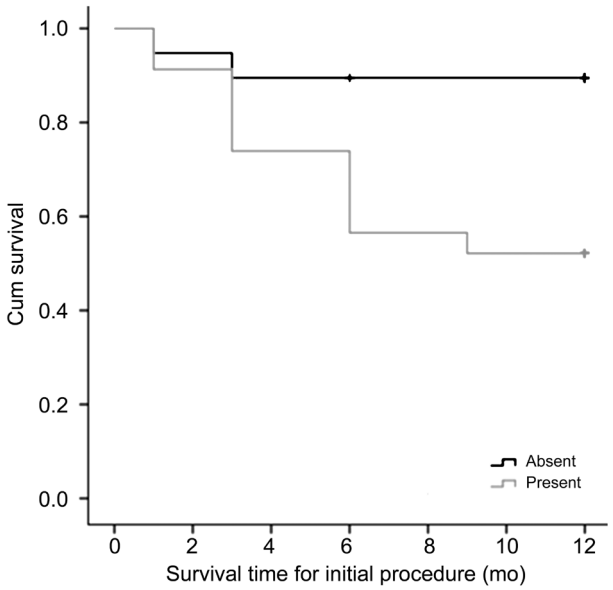

Figure 2 Kaplan-Meier survival analysis: comparing survival time (in months) of the combined procedure in mutation-absent eyes (19 eyes in 15 patients) and mutation-present eyes (23 eyes in 14 patients) subgroups.

The mechanism by which CYP1B1 mutations cause the disease is not fully understood[29]. However, it is hypothesized that these mutations result in either reduced activity or abundance of the CYP1B1 enzyme or both[30]. G61E mutation may be implicated with reduced activity, while frameshift mutations and Y81X mutation can result in a decreased abundance of the protein[30]. The reduction or absence of the cytochrome p450 enzyme 1B1 may affect the spatial and temporal regulation of the development of the anterior chamber angle or lead to arrest of its development through accumulation of toxic metabolites[31]. Hollander *et al*[32] in 2006 studied a small group of PCG patients and classified their angle anomalies histologically into three categories and tried to correlate it with the severity of the disease and the detected CYP1B1 mutations. His work highlighted the possibility that CYP1B1 mutations affect the severity of the disease through affecting the severity of the induced angle anomalies.

Few studies addressed the association of CYP1B1 mutations with poor surgical prognosis in PCG[3,20-21,33-34]. Della Paolera *et al*[20] in 2010 reported that Brazilian patients with CYP1B1 mutations required significantly more surgical procedures to control the IOP than those without mutations. The same finding was reported in a group of Arab and Jewish patients[21]. However, in both of these studies, the surgical procedures performed for the patients were not identified. Chakrabarti *et al*[33] in 2010 analyzed a CYP1B1 promoter polymorphism and studied the survival of IOP control in patients with or without mutations and found a significant difference between the two groups. The mutation group had poorer control of the IOP over the postoperative period of follow-up. These results are in agreement with the current study in which the initial procedure had more tendencies to fail early in the group with confirmed mutations.

Abu-Amero *et al*[3] in 2011 studied a group of 74 eyes of Saudi patients with PCG. They reported a non-significant correlation between success or failure of the surgery with the mutation status. These results are in contrast with the results of the current study. This may be attributed to the fact that their patients were operated without randomization using two different surgical procedures; namely combined trabeculectomy-trabeculotomy and nonpenetrating deep sclerectomy with adjuvant MMC. The later procedure is usually inadequate in presence of an anomalous angle[35].

The success rate of the first procedure in Middle Eastern PCG patients (66%) is lower than Western PCG patients (80%-90%)[36]. Geyer *et al*[21] in 2011 noticed that Jewish PCG patients of European descent had a milder form of the disease and required lesser number of procedure to control the IOP than Arabs and Druze PCG patients. They related this to the lower incidence of CYP1B1 mutations detected in the group of Jewish patients than the other two groups in the study. The success rate in our study was 69% which is comparable to the results reported by Mullaney *et al*[7] in 1999 and Al-Hazmi *et al*[6] in 2005 for combined trabeculotomy-trabeculectomy with MMC in moderate to severe cases (70%-80%). Both studies were carried on Saudi patients who are close in ethnicity to our group of patients.

Overview of the factors related to the success and failure of the surgical procedure in our group revealed that positive consanguinity was significantly associated with more failure of the initial procedure. Furthermore, presence of CYP1B1 mutations, early age at presentation and high initial IOP were all associated with the lower survival and early failure of the initial procedure.

In our cohort, the younger the patient at the initial diagnosis, the higher his presenting IOP and the earlier he might fail the surgery. The early onset and onset at birth has been always related to the poor outcome of the surgery[37-39]. However, in 2005, Levy *et al*[36] underwent a study of prognostic factors for PCG operated in the first 3mo of life in Arab Bedouins. They found that their failure group had significantly higher initial IOP than the successful group, otherwise no other factors; such as mean age at presentation or corneal diameter, was associated with the final outcome. They proposed that initial IOP could be the prognostic factor for the final outcome in PCG. High presenting IOP is considered a severe phenotype that has been associated with presence of CYP1B1 mutations in PCG patients[29,40].

From our results, patients with CYP1B1 mutations were more likely to have lower survival time for the first procedure than the other patients. The patients with mutations had distorted

anatomy at the area of the corneoscleral limbus, which made the identification of Schlemm's canal more difficult and the surgeon noticed increased resistance to the trabeculotome insertion into the canal in this group than the non-mutation group of patients. This observation may signal an under- or abnormal development of Schlemm's canal in these patients. These findings suggest that presence of CYP1B1 could be considered as a prognostic factor for the outcome of the surgery, along with clinical prognostic factors such as poor corneal clarity, high presenting IOP and early onset of the disease. This is especially important in populations with high incidence of CYP1B1 mutations as in the Middle East and India. Belmouden *et al*[26] in 2002 and Abu-Amero *et al*[3] in 2011 proposed screening for the founder mutation, G61E, in PCG patients from Middle Eastern descent due to its high prevalence. However, all sequence alterations of CYP1B1 should be considered.

A limitation of this study may be the small sample size, but the controlled nature of this prospective study compensated for this weakness. All patients were chosen with the same criteria and from the Egyptian population only. In addition, they were operated upon with the same procedure, done by the same surgeon in all patients. This prospective cohort controlled study design is the best to evaluate risk factors and their impact on the natural history of the disease or intervention[41]. The lack of normal control for CYP1B1 mutations and SNPs in the Egyptian population is another limitation, but we compensated for this by comparative sequence alignment with other CYP1B1 orthologous proteins and computer-assisted mutation tolerance simulation. None the less, the screening for SNPs in normal Egyptian population should be considered in further studies. In addition, the presence of compound mutations makes it harder to identify the phenotypic outcome associated with a specific mutation without further investigations. In summary, this study suggests that CYP1B1 mutations participate in the development of primary congenital glaucoma in Egyptian patients. Seven novel CYP1B1 mutations were detected in our group that could be specific to the Egyptian population. Five of these were suggested to be damaging mutations according to "Tolerance Simulation on SIFT". The novel mutation 4545insC resulted in terminating the protein at amino acid 326 eliminating exon 3 from the protein. Further studies are required to determine the effects of these mutations on the function of the protein and consequently the possible role in the development of PCG. On the other hand, G61E mutation was the most frequently encountered in our group with potential adverse effects on the clinical severity and surgical prognosis of PCG. Patients harboring CYP1B1 mutations suffer from early failure and poorer prognosis than patients with no mutations of CYP1B1.

Therefore, CYP1B1 mutations might be considered as a prognostic factor for the surgery in PCG together with clinical prognostic factors. From the outcomes of the current research, we recommend that at least patients with severe phenotype should undergo genetic testing which will give a good prognostic idea regarding the outcomes of their subsequent surgery.

ACKNOWLEDGEMENTS

Clinical part of the work was performed at the Ophthalmology Department, Faculty of Medicine, Cairo University, Egypt. The rest of the work at the Cancer Biology Department, National Cancer Institute, Cairo University, Egypt.

Conflicts of Interest: Khafagy M, None; **El-Guendy N,** None; **Tantawy M,** None; **Eldaly M,** None; **Elhilali H,** None; **Abdel Wahab A,** None.

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