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ORIGINAL ARTICLE

CYP1B1 and myocilin gene mutations in Egyptian patients with primary congenital glaucoma

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Abstract *Purpose:* Primary congenital glaucoma (PCG) accounts for 26–29% of childhood blindness in Egypt. The identification of disease causing mutations has not been extensively investigated. We aimed to examine the frequency of CYP1B1 and MYOC mutations in PCG Egyptian patients, and study a possible genotype/phenotype correlation.

Methods: Ninety-eight patients with PCG diagnosed at the Ophthalmology department of Alexandria Main University Hospital were enrolled. Demographic and phenotypic characteristics were recorded. Patients and 100 healthy subjects (control group) were screened for two mutations in *CYP1B1* gene (G61E, R368H) and one mutation in *MYOC* gene (Gln48His) using polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP). Phenotypic characteristics pertaining to disease severity were compared.

Results: Nineteen patients (19%) with PCG were found positive for one or more of the mutations screened for. Seven patients (7%) were homozygous for the G61E mutation. Ten patients (10%) were heterozygous; 6 for the G61E mutation, 2 for the R368H mutation and 2 for the Gln48His mutation. Two patients (2%) were double heterozygotes harboring a R368H as well as a Gln48His mutation. The most common mutation observed was the G61E in 13 patients; 7 homozygotes and 6 heterozygotes for the mutation. The control group were negative for all mutations screened for.

No significant correlations between the mutations and phenotype severity were detected. A statistically significant positive correlation however was found between the different mutations and each of the IOP and the cup/disk ratio.

Conclusion: The current study further endorses the role of *CYP1B1* mutations in the etiology of PCG among Egyptian patients and is the first study to report *MYOC* gene mutation in Egyptian patients with PCG.

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1. Introduction

Primary congenital glaucoma [PCG (OMIM 231300)] is an autosomal recessive disorder of the eye with an onset in the neonatal or early infantile period. It is caused by developmental defects in the trabecular meshwork and anterior chamber angle resulting in the obstruction of aqueous outflow leading to raised intraocular pressure (IOP) [1]. The increased IOP is responsible for corneal edema, rupture of Descemet's membrane (Haab Striae), and optic nerve damage which if left unattended leads to irreversible blindness [2].

The prevalence of PCG varies across the world with the highest incidence (1:1250) in the Romany population of Slovakia followed by the Middle East (1:2500) and the lowest incidence (1:10,000) in the Western population [3–5].

Genetic heterogeneity is the hallmark of PCG. Four different loci have been identified: GLC3A on 2p21, GLC3B on 1p36.2, GLC3C on 14q24.3 and GLC3D on 14q24 distal to GLC3C (2). To date, three genes have been implicated, and include cytochrome P450, subfamily I, polypeptide 1 (*CYP1B1*), latent transforming growth factor β binding protein 2 (*LTBP2*) and myocilin (*MYOC*) [6]. Mutations in the *CYP1B1* (OMIM 601771), located in the GLC3A locus, is the most common genetic cause of PCG worldwide. In addition, myocilin gene (*MYOC*, OMIM 601652) on 1q23 has been implicated in the pathogenesis of some cases of PCG either independently or in association with *CYP1B1* mutations [7].

PCG accounts for 26–29% of childhood blindness in Egypt [8]. Identification of disease-causing mutations has not been extensively studied. This study was conducted to examine the frequency of CYP1B1 and MYOC mutations in PCG Egyptian patients. In addition, genotype- phenotype correlation was attempted.

2. Patients and methods

The study protocol adhered to the Declaration of Helsinki guidelines and was approved by the Ethics committees of the Faculty of Medicine and the Medical Research Institute, University of Alexandria. An informed consent was signed by the legal guardian of all participants.

2.1. Patients

Ninety-eight patients presenting with and operated upon for PCG at the Ophthalmology department of Alexandria Main University Hospital were enrolled. All patients were examined and operated upon by a single surgeon (NB). A control group of 100 healthy subjects screened for the absence of any ocular disease or abnormality was included for the molecular part of the study. Examination of patients in Alexandria Main University hospital follows a standard protocol previously described by the authors [9,10]. PCG was diagnosed clinically when a patient presented with an enlarged cornea and/or corneal haze with an elevated IOP under inhalational general anesthesia(GA) [11] and optic nerve cupping in the absence of any associated ocular or systemic anomalies or secondary conditions known to be associated with or to cause glaucoma. Owing to the known effect of inhalational anesthetics on measured IOP [12], PCG was diagnosed also if there was a significant difference of more than 5 mmHg in the measured IOP between the 2 eyes [11], or if successive examination under anesthesia (EUA) demonstrated progression in any of measured ocular biometric parameters beyond the normal rate of growth of the eye irrespective of the measured absolute value of the IOP [9–12]. Any patient with a suspiciously low initial IOP under GA was followed up for several weeks to document rapid progression of the cup/disk ratio or the axial length beyond what is expected for that age group. When this was demonstrable, the diagnosis of PCG was established and the patient managed accordingly. Classification of patients into mild, moderate or severe was based on clinical findings [13].

2.2. Molecular study

Peripheral venous blood samples were withdrawn from both 98 patients and 100 healthy volunteer donors (control). DNA extraction was performed using Qiamp DNA Blood Mini kit (Qiagen, Germany) according to the manufacturer's protocol. Screening for 2 mutations in *CYP1B1* (G61E, R368H) and one mutation in *MYOC* (Gln48His) was accomplished using PCR/RFLP. The coding regions (1.6 Kb) spanning exons II and III of *CYP1B1* gene were amplified from genomic DNA of both patients and control samples using three sets of primers as previously described (Table 1) [14,15]. When no mutation was found in *CYP1B1* gene, as well as in patients with heterozygous mutations, amplification of coding region of *MYOC* gene encompassing Gln48His mutation was carried out using previously published primer sequence [7] (Table 1).

2.3. Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS ver.20 Chicago, IL, USA). Comparing quantitative variables between different genetic mutations was done using Kruskal Wallis test. A level of significance of 0.05 was used.

3. Results

The study was conducted on 98 (58 males, 40 females) patients with PCG. Consanguinity was present in 49% of the studied group. A positive family history was present in 12 patients (12%) of the studied group. The demographic and clinical characteristics of the patients are demonstrated in (Table 2).

PCR-RFLP analyses were performed for *CYP1B1* mutations (G61E, R368H) and *MYOC* mutation (Gln48His). Of the 98 patients examined, 19 patients (19%) with PCG were found positive for one or more of the mutations screened for. Seven patients (7%) were homozygous for the G61E mutation. Ten patients (10%) were heterozygous; 6 for the G61E mutation, 2 for the R368H mutation and 2 for the Gln48His mutation. Two patients (2%) were double heterozygotes harboring a R368H as well as a Gln48His mutation.

Among 19 patients carrying a mutation, the most common mutation observed was the G61E present in 13 patients, accounting for 68.5% of mutations screened for. The frequency of the heterozygous R368H mutation, Gln48His mutation was as well as the double heterozygote R368H/Gln48His mutation was 10.5% for each.

Table 1 PCR/RFLP for identification of mutations in *CYPIBI and MYOC* genes.

Gene	Mutation	PCR primers	PCR product (bp)	Restriction enzyme	Digest normal	Digest mutant
CYP1B1	G528A (G61E)	5′-tctccagagagtcagctccg-3′ 5′-gggtcgtcgtggctgtag -3’	786	Taq I	627, 84, 75	318,309,84,75
	G1449A (R368H)	5′-tcccagaaatattaatttagtcactg -3′ 5′-tatggagcacacctcacctg-3′	885	Taa I	507, 200, 178	507, 352, 26
MYOC	G144T (Gln48His)	5′-ggctggctccccagtatatat-3′ 5′-gatgactgacatggcctgg-3′	334	AccI	271, 63	334

CYPIBI: Cytochrome P1B1.
MYOC: Myocilin.
PCR: polymerase chain reaction.
bp: base pair.

Table 2 Demographic data and clinical characteristics of the patients.

The demographic data, clinical characteristics	
	PCG cases
Patients, *n* (%) [98, (100)]	
Male	58 (67)
Female	40 (33)
Eyes, *n* (%) [144, (100)]	
Right eye only	25 (25.5)
Left eye only	27 (27.6)
Bilateral	46 (46.9)
Age at onset of PCG, mean (±SD, range, median) months	3.8 (±4.3, 0–25, 3)
Clinical severity (144 eyes)	
Mild	125 (86.8)
Moderate	19 (13.2)
Severe	0 (0)
Clinical characteristics at presentation mean (± SD, range, median)	
Intraocular pressure (mmHg)	18.1 (±6.3, 4–40, 18)
Corneal diameter (mm)	13.1 (±0.9, 10–16, 13)
Axial length (mm)	23.57 (±2.25, 19.35–32.87, 23.24)
Cup/disk ratio	0.4 (±0.3, 0–1, 0.3)

MYOC gene mutation Gln48His was observed in a heterozygous state in 4 patients of the 91 screened for the mutation (4.4%); 2 of these were double heterozygotes harboring a R368H as well. None of the control group were positive for any of the mutations screened.

The ophthalmologic findings in patients with unilateral/bilateral glaucoma who were positive for one of the mutations screened for are presented in (Tables 3 and 4) respectively.

No significant correlations between the mutations and phenotypic severity were detected. A statistically significant positive correlation however was found between the different mutations and each of the IOP and the cup/disk ratio (Table 5).

4. Discussion

This is the first extensive genetic study demonstrating the role of *CYP1B1* and *MYOC* gene mutations in Egyptian patients with PCG. Our cohort included 98 patients from 98 families as well as 100 control. The patients and control group were screened for 2 mutations in the *CYP1B1* gene which have been implicated in the etiology of PCG in the Arab, Middle Eastern and Mediterranean Countries [16–21]. Several reports have documented the role of *MYOC* gene mutations in the etiology of PCG when no pathogenic mutations were found in the *CYP1B1* gene [21,22]. These reports have prompted us to look for *MYOC* gene mutations in patients who were heterozygous or had no pathogenic mutation in the *CYP1B1* gene.

Screening for mutations in the *CYP1B1* gene and the *MYOC* gene revealed that 19% of the patients enrolled in this study carried a mutation causing PCG. *CYP1B1* is major contributing gene for PCG. In the present study 17% of patients with PCG carried a mutation in the *CYP1B1* gene which is lower than the prevalence of *CYP1B1* mutations reported in Saudi Arabia (79.4%) [16] and Lebanon (33%) [17]. This may be explained by the fact that 82.6% of the patients included in this study had a mild phenotype in contrast to patients from Saudi Arabia and Lebanon who had severe phenotypes. Moreover, forty-two patients (42.8%) in this study had unilateral glaucoma. In a study conducted on cases with unilateral glaucoma in Saudi Arabia, none of the patients were found to harbor a *CYP1B1* gene mutation [18]. Variation in sample size could be another explanation as the Saudi and Lebanese studies included fewer patients than the current study. The presence of disease causing mutations other than those screened for in this study may be another explanation.

Although the frequency of patients with PCG harboring a mutation was lower in the present study compared to other studies, yet, within the group of patients harboring a mutation the frequency of the different mutations screened for was comparable to that reported in the literature. In the present study, the G61E mutation was the most frequently encountered mutation (68.5%) within the group of patients harboring a mutation. This is expected as G61E mutation is a reported founder mutation in Arabs and Middle Eastern populations. The results of the present study are in accordance with the results reported by Badeeb et al. (2014) [16] who found the G61E mutation in 66.6% of Saudi patients harboring a mutation. Moreover, the G61E mutation has been reported to be the commonest mutation in Lebanese patients (33%) [17], Iranian patients (32%) [20] as well as Kuwaiti patients (47%) [19] with PCG.

In the present study R368H mutation was detected in a heterozygous form in four patients (21%) in the mutation positive group. Among patients with PCG harboring a mutation,

Table 3 Ophthalmological findings in patients with unilateral glaucoma positive for a mutation in the CYB1 gene or Myocilin gene.

Patient ID	Age at diagnosis (months)	Sex	Ophthalmology findings					Result of Mutations screened for by PCR/RFLP
			IOP	Corneal diameter	Corneal clarity	Axial length	C/D ratio	
16	2	Male	18	13	Haze	21.44	0.5	Homozygote for G61E
42	1	Female	15	13	Haze	21.12	0.1	Homozygote for G61E
69	2	Female	32	12.5	Edema	21.56	0.5	Homozygote for G61E
51	3	Male	14	12.5	Edema	22.75	0.2	Heterozygote for G61E
68	1	Male	24	14	Haze	22.55	0.6	Heterozygote for G61E
76	2	Male	18	11	Edema	21.67	0.1	Heterozygote for G61E
106	8	Male	24	14	Edema	24.09	0.3	Heterozygote for G61E
56	12	Male	10	13	Clear	22.89	0.1	Heterozygote for R368H
64	6	Male	10	13	Edema	22.28	0	Heterozygote for R368H
47	4	Male	16	13	Edema	23.3	0.1	Heterozygote for Gln48His

Abbreviations: IOP: intraocular pressure, C/D: cup/disk.
PCR: polymerase chain reaction.
RFLP: restriction fragment length polymorphism.

Table 4 Ophthalmological findings in patients with bilateral glaucoma positive for a mutation(s) in the CYB1 gene and/or Myocilin gene.

Patient ID	Age at diagnosis (months)	Sex	Ophthalmology findings										Result of Mutations screened for by PCR/RFLP
			IOP		Corneal diameter		Corneal clarity		Axial Length		C/D Ratio		
			OD	OS	OD	OS	OD	OS	OD	OS	OD	OS	
1	2	Female	18	17	12.5	13.5	Haze	Scar	21.53	23.11	0.9	0.8	Homozygote for G61E
57	11	Male	16	18	14	15	Edema		25.7	26.61	0.8	0.7	Homozygote for G61E
58	2	Male	9	16	14	13	Clear	Hazy	23.08	21.55	0.3	0.3	Homozygote for G61E
97	1	Male	18	24	12	13	Edema		21.54	22.48	0.4	0.4	Homozygote for G61E
71	3	Female	22	18	14	13.5	Haze	Haze	22.45	22.16	0.3	0.3	Heterozygote for G61E
107	1	Male	18	17	12.5	12	Edema		21.09	20.22	0	0.2	Heterozygote for G61E
52	3	Male	4	4	13.5	13	Clear	Clear	25.35	23.28	0.5	0.2	Heterozygote for Gln48His
61	5	Male	17	36	14	14.5	Haab Straie		25.41	25.58	0.8	0.5	Heterozygote for both R368H and Gln48His
70	8	Female	18	26	14	14	Edema		25.01	26.47	0.9	0.9	Heterozygote for both R368H and Gln48His

Abbreviations: IOP: intraocular pressure, OD: right eye, OS: left eye, C/D: cup/disk.
PCR: polymerase chain reaction.
RFLP: restriction fragment length polymorphism.

Table 5 Correlation of mutations with IOP and CD ratio.

	Homozygote G61E	Heterozygote G61E	Heterozygote R386H	Heterozygote Gln48His	Heterozygotes for both R368H and Gln48His	(*p*)
IOP						$p = .02^{*}$
Median	18	18	10	4	22	
(min–max)	9–32	14–24	10–10	4–16	17–36	
C/D ratio						$p = .006^{*}$
Median	0.5	0.25	.05	0.1	0.85	
(min–max)	0.1–0.9	0–0.6	0–0.1	0.1–0.1	0.5–0.9	

By Kruskal Wallis test.
Abbreviations: IOP: intraocular pressure, C/D: cup/disk.
* statistically significant.

Chitsazian et al. 2007 [20] found the R368H mutation in 15% of Iranian patients whereas Giuffre' 2011 [21] detected the R368H mutation in 8% of Italian patients. Although the frequency of the R368H mutation in the present study is higher than that reported in the aforementioned studies, yet, it is quite similar to that reported by Millá et al. 2103 [22] who found that 20% of Spanish patients with PCG possessed the R368H. The North coast of Egypt particularly Alexandria located on the Mediterranean Sea is likely to share genes of other Mediterranean countries, as it was a host to many different communities during the colonial period, including Greeks, Italians, Syrians, Jews and Armenians. The potential role of CYP1B1 has been well documented in PCG cases worldwide. *CYP1B1* gene participates in the normal development and functioning of the eye by metabolizing essential molecules that are probably used in a signaling pathway [23]. Its presence in the inner ciliary and lens epithelia appears to be necessary for normal development of the trabecular meshwork and in regulating intraocular pressure [24]. The highly conserved G61E mutation is adjacent to the N-terminal proline-rich region of *CYP1B1* and is likely to affect the proper protein function and result in disease manifestation [14]. R368H, maps to helix K, which is one of the highly conserved core structures (CCSs). The CCSs are suspected to be involved in proper protein folding and in active heme binding [25].

The presence of mutations in *CYP1B1* gene does not account for all cases with PCG worldwide. Mutations in the *MYOC* gene have been implicated in the etiology of not only POAG (primary open angle glaucoma) [26] and JOAG (juvenile open angle glaucoma) [27] but also in PCG [7]. Four of the ninety-one patients tested (4.4%) were heterozygous for the G144T mutation, of these two were heterozygote for both R368H and Gln48His mutations. This is the first report of *MYOC* gene mutations in Egyptian patients with PCG. *MYOC* gene mutations have been reported as a disease causing mutation in patients with PCG in the Mediterranean region, where Giuffre' 2011 [21] reported that a frequency of 4.2% among Italian patients with PCG and Millá et al. 2103 [22] reported a frequency of 4.8% among Spanish patients with PCG implying that the Egyptian population is an admixture of ethnic background. A potential digenic mechanism in a PCG case resulting from the simultaneous involvement of mutant alleles of *MYOC* and *CYP1B1* has been previously proposed [7]. *MYOC* gene encode myocilin protein, which is found in the trabecular meshwork and the ciliary body of the eye. Mutations in the olfactomedin domain of myocilin (myocOLF) are the strongest link to inherited primary open angle glaucoma. In this recently identified protein misfolding disorder, aggregation-prone disease variants of myocilin hasten glaucoma-associated elevation of intraocular pressure, leading to vision loss [28].

The present study demonstrated no significant correlations between the mutations screened for and phenotypic severity. Our results are in accordance with those of de Melo et al. 2015 [29] who reported a lack of genotype-phenotype correlation of the demographic and clinical traits to *CYP1B1* mutations in PCG. Yet, Panicker et al. 2004 [30] and Berraho et al. 2015 [31] have reported a correlation between disease severity and different pathogenic mutations. In the present study the positive correlation found between the different mutations and each of the IOP and the cup/disk ratio has been previously reported [31,32].

In conclusion, this is the first extensive study addressing the role *CYP1B1* and *MYOC* gene mutations in the etiology of PCG among Egyptians. Our results further endorse the role of *CYP1B1* mutations in the etiology of PCG among Egyptian patients and ours is the first study to report *MYOC* gene mutation in Egyptian patients with PCG. The presence of patients with PCG in whom no mutations in either the *CYP1B1* or *MYOC* genes were detected suggests the presence of other disease causing mutations implying marked genetic heterogeneity of PCG in Egypt.

Conflict of interest

There is no conflict of interest.

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