

Analysis of *CYP1B1* sequence alterations in patients with primary open-angle glaucoma of Saudi origin

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Abstract: Cytochrome P450 Family 1 Subfamily B Member 1 (*CYP1B1*; OMIM# 601771) gene encodes one of the cytochrome P450 family of enzymes. *CYP1B1* mutations have been associated primarily with primary congenital glaucoma (PCG). Similar studies were reported in juvenile open-angle glaucoma, Rieger's and Peters anomalies. Reports of likely pathogenic sequence alterations in families affected with adult-onset primary open-angle glaucoma (POAG) triggered this investigation. We screened unrelated POAG cases and healthy controls for mutations in *CYP1B1* using automated Sanger sequencing to identify five known polymorphisms and one *CYP1B1* mutation (p.G61E) in a heterozygous status. The p.G61E mutation is known to cause PCG in a homozygous or compound heterozygous form, and thus, its presence here in a heterozygous form indicates carrier status. These findings suggest that *CYP1B1* may have no major role in the pathogenesis of POAG, at least, in the Saudi population. However, further investigations are needed to validate these findings in a larger cohort.

Keywords: *CYP1B1*, genetics, mutation screening, POAG, Saudi Arabia

Introduction

Cytochrome P450 Family 1 Subfamily B Member 1 (*CYP1B1*) is a membrane-bound drug-metabolizing enzyme that is also known to be involved in the metabolism of steroids, fatty acids and retinoids that play key roles in cell cycle signaling pathways and apoptosis.¹ *CYP1B1* has been demonstrated to be highly expressed in the iris and ciliary body of the human eye, and plays an important role in the pathogenesis of anterior segment disorders, including glaucoma.¹ There are many postulated theories of how *CYP1B1* may contribute to primary congenital glaucoma (PCG) pathogenesis, including the plausibility of mutations in the *CYP1B1* gene, which may lead to high intraocular pressure (IOP) in the eye due to increased resistance to aqueous humor outflow pathway through anomalous trabecular meshwork development.¹ Mutations in *CYP1B1* have been identified in various ethnicities and different glaucoma phenotypes, some of which are population-specific.^{1,2}

Primary open-angle glaucoma (POAG) is a genetically complex multifactorial trait with the plausibility of many genes contributing directly or indirectly to the development/progression of the disease. The genetic basis of POAG is still obscure, and the various disease-causing genes identified by several genetic investigations so far account for <5% of POAG cases in the general population.³ Among many of these genes identified so far, the presence of *CYP1B1* mutations has also been reported in families where PCG and POAG coexist, indicating the existence of common or overlapping

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CYP1B1-mediated pathophysiological mechanism(s) in these two forms of glaucoma.⁴ Besides, *CYP1B1* has been previously indicated for its plausible causative role in early-onset POAG patients from France,⁵ Germany⁶ and Spain.⁷ Furthermore, in vitro functional assay demonstrated that these mutations exhibited none or reduced enzymatic activity, to be classified as null or hypomorphic alleles, respectively, indicating *CYP1B1* mutations to be a risk factor for POAG.^{6,8} In the present study, we investigated whether POAG patients from Saudi Arabia harbor mutation(s) in the *CYP1B1* gene.

Patients and methods

The well-defined clinically diagnosed POAG patients (n=50) and healthy controls (n=50) of Saudi origin were recruited to the study at King Abdulaziz University Hospital in Riyadh, Saudi Arabia. POAG patients satisfied the following clinical diagnostic criteria: 1) presence of the disk or retinal nerve fiber layer (RNFL), eg, thinning or notching of disk rim, progressive changes and nerve fiber layer defect; 2) presence of visual field abnormalities (eg, arcuate scotoma, nasal step, paracentral scotoma and generalized depression) in the absence of other causes or explanation; and 3) open angles bilaterally on gonioscopy. Cases with evidence of any secondary form of glaucoma, eg, pigmentary glaucoma, uveitic, pseudoexfoliation and history of steroid use or ocular trauma were excluded. Ethically matched control subjects were selected from ophthalmic screening clinics and individuals with normal IOP (without any anti-glaucoma medication), open angles on gonioscopy and healthy optic disk on examination with no history of ocular disease(s) or previous ophthalmic surgeries. Individuals refusing to participate in the study were excluded. History of the presence or absence of systemic diseases, such as diabetes mellitus, hypertension, coronary artery disease and hypercholesterolemia, and smoking status was procured through medical records of the patients or personal interviews for controls.

Polymerase chain reaction-based automated Sanger sequencing was used to screen for *CYP1B1* mutations following the protocol described elsewhere.² PolyPhen-2 online tool (<http://genetics.bwh.harvard.edu/pph2/>) was used to assess the possible (damaging/benign) effect of the sequence alterations on the protein structure and/or function as described previously.²

Statistical analysis

Data were represented as mean \pm SD for continuous variables and as frequency and percentage for nominal variables. Nominal variables were tested by Pearson's Chi-square test

or Fisher's exact test (as indicated). Independent samples *t*-test was used to detect the mean difference across groups. A *p*-value <0.05 was considered statistically significant. SPSS version 22 (IBM Corporation, Armonk, NY, USA) was used to perform the analyses.

Ethics approval and consent to participate

The study adhered to the tenets of the Declaration of Helsinki and received approval from the institutional review board and research ethics committee of College of Medicine, King Saud University, Riyadh, Saudi Arabia (approval number # 08-657). Written informed consent was obtained from all participants prior to their inclusion in this study.

Results

Table 1 shows the demographic and clinical characteristics of patients and controls selected in this study. Except for family history of glaucoma, there was no significant difference between patients and control groups for age, gender, smoking habit and other systemic co-morbidities.

CYP1B1 sequencing revealed a total of five previously reported single nucleotide polymorphisms (SNPs): R48G (rs10012), A119S (rs1056827), L432V (rs1056836), D449D (rs1056837) and N453S (rs1800440). Additionally, we detected one *CYP1B1* mutation (G61E) in a heterozygous form as shown in Table 2. Previously, it was reported that the p.G61E mutation was pathogenic in the Saudi PCG patients in a homozygous form.² However, in this study, the mutation was detected in heterozygous form and accounted for 4% and 2% of the POAG cases and controls, respectively. None of the variants showed any significant association with POAG as compared to controls.

Discussion

Genome-wide and candidate gene association studies have so far identified 66 loci harboring 76 different genes in POAG with conflicting findings.³ Many of these studies support the hypothesis that *CYP1B1* has a broader significance in POAG.¹ However, two meta-analysis studies have found no association between *CYP1B1* variants and POAG.^{9,10} In accordance with these studies, our study also reports a negative association between *CYP1B1* and POAG.

Heterozygous *CYP1B1* mutations have been reported in French,⁵ German⁶ and Spanish⁷ POAG patients. These mutations have been functionally demonstrated to be hypomorphic or null alleles.^{6,8} However, heterozygous *CYP1B1* variants have also been reported in healthy subjects.⁵⁻⁷ Even in this

Table 1 Demographic and clinical characteristics of POAG cases and controls included in this study

Variables	POAG (n=50) No (%)	Controls (n=50) No (%)	p-value ^a
Demographic characteristics			
Age in years, mean (±SD)	59.8 (11.5)	60.2 (8.2)	0.817 ^b
Male/female	31/19	29/21	0.680
Systemic diseases			
Diabetes mellitus	21 (42.0)	24 (48)	0.548
Coronary artery disease	2 (4.0)	1 (2.0)	0.999 ^c
Hypertension	17 (34)	14 (28.0)	0.516
Hypercholesterolemia	4 (8.0)	2 (4.0)	0.677 ^c
Health awareness/behavior			
Family history of glaucoma	8 (16.0)	1 (2.0)	0.030 ^c
Smoking	3 (6.0)	4 (8.0)	0.999 ^c
Glaucoma indices			
Intraocular pressure in mmHg, mean (SD)	22.0 (8.2)	NA	–
Cup/disk ratio	0.84 (0.6)	NA	–
Number of anti-glaucoma medications	1.7 (1.1)	0 (0)	–
LogMAR visual acuity, mean (±SD)	0.60 (0.77)	NA	–

Notes: ^aPearson Chi-square test. ^bIndependent samples t-test. ^cFisher's exact test.

Abbreviations: LogMAR, Logarithm of the minimum angle of resolution; NA, not available; POAG, primary open-angle glaucoma.

study we identified a heterozygous p.G61E mutation in *CYP1B1* in two POAG patients and one healthy control, which raises questions about its role as a disease-causing mutation.

The gene for *CYP1B1* is localized to chromosome 2 and consists of three exons, of which exons 2 and 3 encode the functional protein suggested to be involved in IOP regulation.¹ The exact role of *CYP1B1* in POAG and/or ocular development is still lacking. However, recent functional studies on characterization of a common homozygous (p.G61E) *CYP1B1* mutation in a glaucoma family have demonstrated that this mutation affects the extracellular matrix (ECM) microfibrils, making it less abundant and more fragmented in affected individuals. The authors suggested that disruption of ECM-trabecular meshwork caused increased resistance to aqueous outflow leading to elevated IOP and subsequent development of glaucoma.¹¹ Similarly, missense *CYP1B1* mutations reported to be associated with POAG were

demonstrated to affect the metabolism of 17β-estradiol, which in turn were implicated to contribute to POAG pathogenesis through *MYOC* (OMIM# 601652) overexpression or increased oxidative stress and apoptosis.¹²

Interestingly, our POAG cohorts were previously screened for *MYOC* and *OPTN* genes and found to have no mutation(s) in both these genes.¹³ Taken together, the absence of mutations in the *CYP1B1* gene in the Saudi POAG patients suggests that *CYP1B1* mutations do not contribute to POAG pathogenesis in the Saudi population. Our finding supports the conclusion reached by the recently conducted meta-analysis studies.^{9,10} However, as with other complex diseases, including POAG, large sample numbers are needed to ensure sufficient power to identify the underlying genetic causal effect which may be a limiting factor in this study; besides, the presence of rare variants cannot be ruled out in this study, and as such, these findings need further validation in a larger cohort.

Table 2 Analysis of the *CYP1B1* sequence changes detected in the POAG patients

Nucleotide change	AA change	Mutation type	Homozygous/ heterozygous	% in POAG ^a	% in controls	Pathogenicity assessment
g.3947C>G	R48G	Missense	Heterozygous	56	56	Polymorphic
g.3987G>A	G61E	Missense	Heterozygous	4	2	Pathogenic ^b
g.4160G>T	A119S	Missense	Heterozygous	38	38	Polymorphic
g.8131C>G	L432V	Missense	Homozygous	60	62	Polymorphic
g.8184C>T	D449D	Silent	Homozygous	58	62	Silent
g.8195A>G	N453S	Missense	Homozygous	22	22	Polymorphic

Notes: ^aNon-significant ($p > 0.05$) as compared to controls (Fisher's exact test). ^bThis mutation is causative if inherited in homozygous form.

Abbreviations: *CYP1B1*, Cytochrome P450 Family 1 Subfamily B Member 1; POAG, primary open-angle glaucoma.

In the past 9 years, we have investigated various SNPs with their possible association with POAG in the Saudi population. Among these, we have so far found only two SNPs that were associated with POAG. The first was a “T” allele in the *SIX1/SIX6* gene¹⁴ and second was an SNP in the *GST* gene.³ Thus, as far as the genetic cause for POAG in the Saudi population, the jury is still out.

Conclusion

No significant distribution of *CYP1B1* mutations or polymorphisms was found in our POAG cohort, indicating that *CYP1B1* may not have a major role in POAG, at least, in Saudi population. However, further investigations are needed to confirm these findings.

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Author contributions

All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflict of interests in this work.

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