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CYP1B1 Gene and Phenotypic Correlation in Patients From Northeastern Brazil With Primary Congenital Glaucoma

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Purpose: To identify variants in the *CYP1B1* gene in northeastern Brazilian patients with primary congenital glaucoma (PCG) and possible genotype-phenotype correlations.

Materials and Methods: This is a cross-sectional observational study of 17 nonrelated patients with PCG, performed at the Altino Ventura Foundation, Recife, Brazil, between December 2017 and February 2018. All patients underwent an examination, including gathering information from their medical records, slit-lamp examination, fundoscopy, tonography, and measuring corneal diameter and thickness.

Results: The mean age at the time of the examination was 27.7 years; 52.9% (n = 9) were male, 29.4% (n = 5) had history of parental consanguinity. The mean age when the diagnosis was confirmed was 0.53 ± 2.18 years. Horizontal corneal diameter ranged from 12 to 16 mm (mean: 14.05 ± 1.42 mm) and the IOP mean value was 17.31 ± 9.84 mm Hg. Predicted pathogenic variants of the *CYP1B1* gene were identified in 4 patients (23.5%). The differences among all clinical parameters did not reach statistical significance between individuals with and without *CYP1B1* variants (*P*-values > 0.05).

Conclusions: Two variants which had not been previously related to PCG in Brazil (c.182G > A, c.241T > A) were identified. No statistically significant genotype-phenotype correlations were found.

Key Words: primary congenital glaucoma, blindness, genotype, phenotype, *CYP1B1* gene

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Primary congenital glaucoma (PCG)[1] is a rare and severe genetic disease which is commonly expressed during the first year of life and represents an important cause of childhood blindness worldwide.[2] The disease is characterized by increased intraocular pressure (IOP) which results in damage to the optic nerve. This increase in the IOP is secondary to an obstruction of the aqueous humor drainage, which is due to a malformation in the trabecular meshwork.[3–5] Furthermore, edema, corneal opacity, corneal enlargement, Descemet membrane rupture (Haab's Striae), buphthalmos, refractive errors, photophobia, epiphora, and blepharospasm are some other clinical features of PCG.[6,7]

PCG is the most common type of glaucoma in childhood, with a global incidence rate of ~1:10.000 births.[8] This disease exhibits a high prevalence in populations where consanguinity is common,[9] such as in Saudi Arabia (1:2500)[6] and among a population in southern India (1:3.300).[10]

PCG inheritance is primarily autosomal recessive, with 10% to 40% of its cases being familial.[11,12] Wiggs et al[12] suggested that the number of individuals with *CYP1B1* deleterious variants might be higher than what was expected among the US population.

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).[13] Nevertheless, only 3 genes were found to be involved in the development of PCG: *CYP1B1* (cytochrome P450, family 1, subfamily B, polypeptide 1), situated in the GLC3A locus; *LTBP2* (latent protein-binding factor of beta 2 growth), located in the GLC3D; and *TEK* (tyrosine kinase receptor), in the GLC3E. The role that the proteins encoded by these genes play in the disease etiology remains unclear.[13–15]

It is important to emphasize that > 140 variants in the coding sequence of the *CYP1B1* gene related to the development of PCG have already been reported.[16,17]

Because of the fact that PCG presents a phenotypic heterogeneity, we believe that ethnically diversified populations, such as the Northeastern Brazilian population, might experience important improvements by obtaining early diagnosis and treatment. This would contribute to lower rates of unnecessary blindness.

MATERIALS AND METHODS

This was a cross-sectional observational study of 17 nonrelated Brazilian patients with PCG diagnosis, whose follow-up was performed at the Altino Ventura Foundation (Recife, Pernambuco, Brazil). This study was realized within the period between December 2017 and February 2018.

The study aims to determine the frequency and variant types of *CYP1B1* in patients from northeastern Brazil with the PCG diagnosis and try to establish a correlation between alterations in *CYP1B1* sequencing and the disease phenotype with regard to the clinical outcomes of PCG.

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All patients underwent an ophthalmologic examination through measuring visual acuity, slit-lamp examination, fundoscopy, measuring IOP through a Goldman or Perkins tonometer, corneal pachymetry, and measuring the corneal diameter. Visual acuity was measured using the Snellen chart at 20 feet of distance. The ultrasonic pachymetry was performed using a DGH4000B Pachymeter (DGH Technology Inc., Exton, PA). Ten consecutive measurements were performed in each eye after administering topical ophthalmic anesthetic. The corneal diameters were measured by an ophthalmic compass. Personal information such as sex, family consanguinity, age at disease onset, glaucoma medications and the number of previous ocular surgeries were obtained during the medical interview.

Patients with compatible findings of secondary glaucoma and/or any other ocular or systemic disease that could lead to optic disc abnormalities simulating glaucomatous lesions were excluded from the study. The patients were selected without any sex or age distinctions.

This study followed the tenets of the Declaration of Helsinki and all participants or their legal persons signed the free and informed consent form. This research project was approved by the Ethics Committee for Research involving human beings of the Federal University of Pernambuco's Center of Health Sciences (N. 1.783.121). The authors declare that they have no conflicts of interests, and informed consent was obtained from all individual participants included in the study.

Analysis of *CYP1B1* Gene

Genomic DNA was isolated from peripheral blood samples of all patients. The genomic DNA was amplified by polymerase chain reaction (PCR) with GoTaq Green Master Mix reagent 2× (Promega, Madison, WI) according to vendor specifications, using 3 pairs of specific primers corresponding to the coding sequence and intronic flanking region of *CYP1B1* exons. After confirming the success of the 1% agarose gel amplification, the PCR products of each patient were mixed in a single tube (performing a pool) and quantified by Qubit 2.0 equipment. Each pool of different patients was prepared for second-generation sequencing using a Nextera XT DNA Library Prep Kit (Illumina) that works from the PCR product by tagging and adding adapters, according to the protocol provided by the manufacturer. The second-generation sequencing, also known as next generation sequencing (NGS), was performed in Illumina MiSeq equipment (Illumina, San Diego), and uses the sequencing technology of each amplicon by synthesis in a MiSeq Reagent Kit V2 kit (composed of enzymes, dNTPs, fluorescently labeled ddNTPs and buffers) according to what is recommended by the manufacturer. After the result is acquired by the equipment, the raw data is processed by specific bioinformatics tools generating individual files per patient pool. The use of internal and external quality controls ensures that all steps from sample preparation to release analysis have yielded reliable results. The produced sequences were processed by a bioinformatics team and transformed into analyzable data.

The primer sequences used for *CYP1B1* variants analysis were: CCCAGAGGCTGGGGTAG, CCTCGGG TCGAGGAAGG, AACTTCTTCACGCGCCAG, AAAC TCAGCATATTCTGTCTCTACTCC, AATGGGAAAG ACAGCATTAGTC, ATGAAGAACCGCTGGGTATG, GCTGGGATTACAAGCCTAAGG, GAAGAAACTCC CACCACAGC, GCGGTAGAGACCCGGAC, GGGAG CCGTTTTAGCCC, AATGCACTTTGCCCTCTCC, GA CAAGCTCCGATGCCC.

Genotype-phenotype Correlation

A comparison was performed between the clinical findings of patients with *CYP1B1* gene variants and patients without an identified variant in order to study the relation 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, number of surgical interventions, number of antiglaucoma medications, corneal haze, Haab's striae, buphthalmos, cup/disc ratio, sex, blindness, and consanguinity.

Statistical Analysis

A spreadsheet in Microsoft Excel was created in order to analyze the data, which was then transferred into SPSS version 18 software to perform the analysis. Mann-Whitney *U* test was performed to compare the means of the 2 independent samples, and Fisher exact test was performed to compare categorical variables. A *P*-value <0.05 was used for evidence of statistical significance.

RESULTS

Eight (47.06%) of the 17 patients were female and 9 (52.94%) were male. Among these patients, 15 (88.24%) showed bilateral impairments. The age when the diagnosis was confirmed varied from 0 to 9 (mean 0.53 ± 2.18 y). In the clinical evaluation, horizontal corneal diameter ranged from 12 to 16 mm (mean 14.05 ± 1.42 mm), and the mean IOP value was 17.31 ± 9.84 mm Hg. At least 2 surgical procedures in 18 eyes (52.9%) were required.

Predicted pathogenic variants of the *CYP1B1* gene were identified in 4 patients (23.5%). Most of these were missense variants: c.182G > A, c.241T > A, and c.1310C > T. One nonsense (c.55C > T) and 1 frameshift (c.1209_1210ins TCATGCCACC) were also detected. We also found 1 case of homozygosity (Table 1). Nine single nucleotide variants

TABLE 1. CYP1B1 Gene Variants Identified in Patients From Northeastern of Brazil With the Diagnosis of PCG

Patients	Nucleotide Change	Protein Change	Exon	Zigozity
RC09	c.55C > T	p.Gln19Ter	2nd exon	Heterozygous
	c.241T > A	p.Tyr81Asn	2nd exon	Heterozygous
RC15	c.1310C > T	p.Pro437Leu	3rd exon	Heterozygous
	c.1209_1210insTCAT GCCACC	p.Thr404SerfsTer30	3rd exon	Heterozygous
RC16	c.1310C > T	p.Pro437Leu	3rd exon	Homozygous
RC17	c.1209_1210insTCATGCCACC	p.Thr404SerfsTer30	3rd exon	Heterozygous
	c.182G > A	p.Gly61Glu	2nd exon	Heterozygous

PCG indicates primary congenital glaucoma.

(SNV) were reported: c.-1-12C > T (intron 1—rs2617266); c.1-14C > T (intron 1—rs4987134); c.1044-141_1044-140insT (intron 2—rs34468862); c.142C > G (R48G-exon 2—rs10012); c.355G > T (A119S—exon 2—rs1056827); c.729G > C (V243V—exon 2—rs9341249); c.1294C > G (L432V—exon 3—rs1056836); c.1347T > C (D449D—exon 3—rs1056837) and c.1358A > G (N453S—exon 3—rs1800440) (dbSNP—www.ncbi.nlm.nih.gov/SNP/).

All patients (100%) in the group with variants developed bilateral PCG, and 11 patients (84.6%) in the group without variants expressed bilateral disease ($P = 1.000$). However, the differences between individuals with and without CYP1B1 variants did not reach statistical significance for any of the variables (P-values > 0.05 in all comparisons) (Table 2).

DISCUSSION

CYP1B1 variants were identified in 4 patients (23.53%), and only one among these was homozygous. Five different variant types were detected, and all of them had already been described in PCG patients from different populations,[18–22] although c.182G > A and c.241T > A have never been cited in Brazilian patients.

The prevalence of *CYP1B1* variants that were found in the present study (23.53%) is comparable to what was observed in other populations such as in Mexico (10%), Japan (20%), and Portugal (28.57%).[23–25] In contrast, there is a discrepancy between these numbers and those obtained in populations with high consanguinity rates such as in Morocco (40%), Iran (70%), Kuwait (70.6%), and Saudi Arabia.[6,26–28] Different results were found in previous Brazilian studies such as those performed by De Melo et al[29] and Della Paolera et al[30] with 44% and 30%, respectively. Nevertheless, these studies did not include patients from northeastern Brazil in their samples, which is the object of the present study.

The 6 SNVs identified in exons in this study do not alter the disease phenotype and were previously described in affected individuals and healthy controls. Likewise, 3 SNVs were found in introns; 2 of them (rs4987134 and rs34468862) had no citation found in the literature, but the other one (rs2617266) was already described.[31]

Almost all *CYP1B1* gene variants identified in this study were heterozygous, showing that there is an allelic heterogeneity in patients with PCG from Northeastern Brazil, which is in accordance with the low consanguinity frequency in this region.

Martin et al[32] speculated that individuals with compound heterozygous *CYP1B1* gene variants may exhibit a less severe form of the disease than those with homozygous alterations. On the other hand, in a cohort study, Lim et al[33] observed that both compound heterozygous and homozygous cases had indiscriminate clinical courses of the disease. This divergence in results is not well explained and could possibly be a consequence of regional differences, consanguine marriage percentage, variety of surgical techniques, immediate access to health services, postoperative follow-up time, population composition, studied samples or other factors not yet established.

Regarding the genotype-phenotype correlation, a great variability was shown in relation to the *CYP1B1* gene and the PCG. Weisschuh et al[34] compared a group of PCG patients with *CYP1B1* variants with a group of patients without *CYP1B1* variants, and they did not observe significant differences between them regarding the age of disease onset, the severity of the condition or the response to the treatment. Campos-Mollo et al[21] did not show significant differences between the presence or not of variants in relation to the ocular involvement, age at diagnosis, sex or number of surgical interventions. However, Abu-Amero et al[28] observed that individuals with pathogenic variants of *CYP1B1* had higher rates of postoperative visits and a greater necessity of antiglaucomatous drugs than individuals without pathogenic variants.

The present study did not find significant differences in the phenotype among the PCG population, with and without *CYP1B1* variants, although the limited costs of the research and consequently the limited number

TABLE 2. Clinical Parameters and Demographic Profile of Patients From Northeastern of Brazil With PCG and Variants of the *CYP1B1* Gene and Patients Without Identified *CYP1B1* Gene Variants

Clinical Parameters	Patients With Variants (n = 4)	Patients Without Variants (n = 13)	*P*
Sex [n (%)]			
Female	3 (75.0)	5 (38.5)	0.294†
Male	1 (25.0)	8 (61.5)	
Consanguinity [n (%)]	2 (50.0)	3 (23.1)	0.538†
Age at diagnosis (y)	2.3 ± 4.5	0.00 ± 0.00	—*
Age at evaluation (y)	25.2 ± 15.6	28.5 ± 10.5	0.071*
No. antiglaucoma medications required	3.5 ± 1.0	2.5 ± 1.3	0.111*
IOP at evaluation (mm Hg) (RE/LE)	15.0 ± 2.4/12.5 ± 3.5	19.3 ± 9.7/16.6 ± 12.6	0.569/0.746*
Blindness in at least one eye	75.0	91.7	0.450†
Horizontal corneal diameter (mm) (RE/LE)	13.6 ± 1.3/13.5 ± 2.1	14.0 ± 1.4/14.3 ± 1.6	0.605/0.478*
Corneal thickness (μm) (RE/LE)	524.3 ± 20.6/523.0 ± 17.0	598.4 ± 111.0/557.6 ± 117.9	0.117/ 0.814*
No. surgical interventions (RE/LE) [n (%)]			
0-1	1 (25.0)/3 (75.0)	6 (46.2)/6 (46.2)	0.603/0.576†
≥ 2	3 (75.0)/1 (25.0)	7 (53.8)/7 (53.8)	
Corneal haze (RE/LE) [n (%)]	1 (25.0)/0 (0,0)	5 (38.5)/4 (30.8)	1.000/0.519†
Haab' strie (RE/LE) [n (%)]	0 (0.0)/0 (0.0)	3 (23.1)/6 (46.2)	0.541/0.237†
Buphthalmos (RE/LE) [n (%)]	1 (25.0)/0 (0.0)	4 (30.8)/4 (30.8)	1.000/0.519†
Cup/disc ratio (RE/LE)	0.5 ± 0.4/0. ± 0.4	0.8 ± 0.2/0.9 ± 0.3	0.404/0.073*

Values are mean ± SD.
*Mann-Whitney Test.
†Fisher Exact Test.
IOP indicates intraocular pressure; PCG, primary congenital glaucoma.

of participants may have contributed to this conclusion. Other studies have reported similar results.[6,29]

The major limitation of this study was the small sample size due to the high costs of genetic testing. Nevertheless, it showed important results. Two variants that had not been previously related to PCG in Brazil (c.182G > A, c.241T > A) were identified.

In this study, there were no statistically significant differences between the clinical findings of the cases with and without variants. In addition, knowledge about PCG genetics is still far from complete and remains to be a challenging subject for further research. Therefore, early recognition of PCG signs and symptoms, and identification of families bearing pathogenic variants might have a significant impact on the prediction of disease severity and may help predict surgical outcomes. More efforts are needed to provide effectiveness, timely screening, and appropriate allocation of resources to enable health professionals to reduce the rates of avoidable blindness in Brazil and worldwide.

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