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Association between *CYP1A1*m1 gene polymorphism and primary open-angle glaucoma

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ABSTRACT. The *CYP1A1* gene is related to the generation of secondary metabolites that are capable of inducing DNA damage. The *CYP1A1*m1 polymorphism has been examined in many studies, and is located in a region near loci that have been linked to glaucoma, including the locus *GLC1I*. As a result, this polymorphism has been related to several diseases that are influenced by exposure to xenobiotic as well as primary open-angle glaucoma. We compared the prevalence of the *CYP1A1*m1 polymorphism in 152 Brazilian patients, 100 patients with primary open-angle glaucoma, and 52 normal controls using restriction fragment length polymorphism analysis. The frequency of the homozygous wild-type (w1/w1) *CYP1A1* gene among patients with primary open-angle glaucoma (N = 100) was 16%, for genotype w1/m1, the frequency was 77%, and for m1/m1 it was 7%. Among the control group (N = 52), the frequency of the homozygous wild-type (w1/w1) *CYP1A1* gene was 54%, the frequency of w1/m1 was 46%, and the frequency of m1/m1 was 0%. The presence of the *CYP1A1*m1 polymorphism may interfere with xenobiotic metabolism and exacerbate direct or indirect damage to the optic nerve. These *CYP1A1*m1

polymorphisms may be risk factors for primary open-angle glaucoma.

Key words: *CYP1A1*; *CYP1A1*m1; Glaucoma; Polymorphism; Primary open-angle glaucoma; Restriction fragment length polymorphism

INTRODUCTION

Glaucoma is a complex and genetically heterogeneous optic neuropathy that includes a number of eye diseases characterized by progressive death of retinal ganglion cells through apoptosis. The process is responsible for progressive damage to the optic nerve and loss of vision (Quigley et al., 1995). Glaucoma can be divided into 4 main groups: 1) primary open-angle glaucoma (POAG), which has the highest incidence rate; 2) angle-closure glaucoma; 3) combined glaucoma; and 4) congenital glaucoma (Stamper et al., 1999). According to the age at onset of glaucoma, POAG can be classified into juvenile primary open-angle glaucoma and adult-onset primary open-angle glaucoma (Johnson et al., 1996).

Intraocular pressure is an important risk factor for POAG (Stewart et al., 2000). It is more prevalent, more serious, and shows the worst prognosis in the black ethnic group (Tielsch et al., 1994). Heredity, cardiovascular diseases, exfoliation syndrome, and pigment dispersion syndrome are also considered risk factors for POAG (Stewart et al., 2000). Both the incidence and prevalence of the disease increase with age (Quigley and Vitale, 1997). Four genes have been found to be associated with POAG in the 22 loci reported. These genes include *MYOC*/*TIGR*, *OPTN*, and *WDR36* related to adult-onset primary open-angle glaucoma, and *CYP1B1* related to juvenile primary open-angle glaucoma (Stone et al., 1997; Stoilov et al., 1997; Rezaie et al., 2002; Monemi et al., 2005).

The gene *MYOC* (1q24.3-q25.2, the *GLC1A* locus) was the first glaucoma-related gene to be identified (Stone et al., 1997). The *OPTN* gene was identified in chromosome 10p14-p15, and causes normal-pressure glaucoma, which is a subtype of POAG (Stoilov et al., 1997). A new locus related to POAG (*GLC1G*) and the gene that causes the disease, *WDR36* in chromosome 5q21.3-q22.1, were identified by Monemi et al. (2005). Mutations in *CYP1B1* (2p22-p21, the *GLC3A* locus) are likely to cause juvenile primary open-angle glaucoma or risk factors for the disease (Rezaie et al., 2002). Silva et al. (2009) found no association between the codon 72 polymorphism in the *Tp53* gene and POAG, but some studies have identified differences (Lin et al., 2002). In 2012, Silva (unpublished data) noted a statistically significant relationship between POAG and the *GSTM1* null polymorphism, but no significant relationship was found between these factors. Barbosa et al. (2012) found no statistically significant relationship between POAG and *GSTM1*.

Each day, humans are exposed to numerous compounds that are foreign to the body, known as xenobiotics; their elimination from the body depends on a process referred to as biotransformation (Franco and Franco, 2003). The xenobiotic-metabolizing machinery is formed by phase I and phase II enzymes. Many external compounds are converted into highly reactive metabolites by phase I oxidative enzymes, particularly the cytochrome p450 gene superfamily (CYP). Phase II reactions are established by conjugation with an endogenous substrate through glutathione S-transferase, UDP glucuronyl transferase, and *N*-acetyltransferase, which inactivate products of phase I by converting them into hydrophilic metabolites that are easily excreted from the body (Rossit and Conforti-Froes, 2000).

CYP1A1 is among the most important genes in the *CYP1* family and is related to the generation of secondary metabolites, which are capable of inducing DNA damage. The *CYP1A1* gene is located on chromosome 15q22-q24 and is composed of 7 exons (Corchero et al., 2001). Two different polymorphisms have been identified to be associated with *CYP1A1*. First, the m1 polymorphism (*CYP1A1*m1) or *CYP1A1**2A is characterized by a T to C transition in exon 7 in the 3' non-coding region, where it eventually establishes a cleavage site (*Msp*I restriction enzyme site). Second, the m2 polymorphism, characterized by an A to G transition, leads to an amino acid substitution from valine to isoleucine at codon 462 (Slattery et al., 2004).

Locus *GLC1I* has been linked to glaucoma and is located on chromosome 15q11-13. This region is close to the polymorphism *CYP1A1*m1 (15q22-24), which has been widely examined and related to several diseases that are influenced by exposure to xenobiotics as well as POAG (Mahdy, 2010).

The identification of new modifier genes that may contribute either to the onset of disease or to decreasing disease severity is very important. These studies increase the understanding of the mechanisms by which glaucoma develops, enable better treatment, and improve diagnostic methods. This is important for avoiding a larger socioeconomic impact. In this study, we investigated the frequency of genotypes (w1/w1, w1/m1, or m1/m1) in the *CYP1A1*m1 polymorphism in a POAG patient group and a control group. We analyzed whether the *CYP1A1*m1 polymorphism is correlated with age, gender, ethnicity, and smoking.

MATERIAL AND METHODS

The present study was a retrospective case-control study. We collected 10 mL peripheral blood from 152 patients; within this group, 100 were diagnosed with primary open-angle glaucoma and 52 patients were glaucoma-free, and thus were included in the control group. Patients were treated at Pronto Eye Clinic in Goiânia - Goiás, Brazil. The study was reviewed and approved by the Pontifícia Universidade Católica de Goiás Research Ethics Committee. All patients answered a socioeconomic questionnaire and all data concerning the patients were collected in appropriate forms along with informed consent. All patients underwent complete ophthalmic examination, including a visual acuity test (testing for both corrected and uncorrected vision), gonioscopy, biomicroscopy, funduscopy, tonometry using a Goldmann applanation tonometer, Humphrey automated perimetry using the 24-2 SITA Standard test procedure, retinal angiography (optical disc), and corneal pachymetry.

The study group inclusion criteria were: 60% of optic disc excavation, intraocular pressure higher than 21 mmHg; alteration of the visual field suggesting glaucoma damage; biomicroscopy showing no infection; cataract surgery or previous eye surgery; and Shaffer gonioscopy (grade 4, which means that it would be possible to analyze all camerular gulf structures). The control group inclusion criteria were: optic disc excavation ≤ 50% and lack of asymmetry; intraocular pressure lower than 21 mmHg; normal visual field and biomicroscopy; no previous eye surgery, and no wide camerular angle (Shaffer grade 4) (Susanna, 1998; Stamper et al., 1999).

To extract genomic DNA, we used the Ilustra GFX kit (GE Healthcare; Little Chalfont, UK). The DNA samples were subjected to polymerase chain reaction to amplify the *CYP1A1* gene. The gene *ZFX/Y* was used as an internal control for human DNA and the nucleotide sequences used were previously described by Mota et al. (2010) and Simoni et al. (1999). All procedures used to analyze both genes were performed in duplicate.

The polymerase chain reaction-restriction fragment length polymorphism technique

was used to detect the *CYP1A1*m1 polymorphism. Amplified fragments were analyzed by enzymatic digestion using the restriction enzyme *Msp*I (Fermentas; Vilnius, Lithuania) according to manufacturer instructions. The fragments were analyzed on a 2% agarose gel and stained with ethidium bromide. The wild-type allele (w1) showed a single band of 340 base pairs (bp), the heterozygote (w1/m1) has 3 different fragments, 340, 200, and 140 bp, and the mutant homozygote (m1/m1) showed 2 fragments of 200 and 140 bp.

The chi-squared statistic test (χ^2) was used to compare the distribution of different genotypes from the case and control groups. The Biostat version 5.0 software was used for statistical analysis. The probability of the association between the characteristics analyzed was calculated by Fisher's test. The odds ratio (OR) and 95% confidence interval (95%CI) were calculated to evaluate the degree of association between the glaucoma and control groups. P-values lower than 0.05 were considered to be statistically significant.

RESULTS

The frequency of the alleles (w1/w1, w1/m1, and m1/m1) was assessed for the glaucoma and control groups. The frequency of genotype w1/w1 was approximately 3-fold higher in the control group (54%) compared to the glaucoma group (16%) (Table 1).

Table 1. Frequency of the genotypes w1/w1, w1/m1, and m1/m1 of the *CYP1A1* gene between the glaucoma and control groups.

Genotype	Glaucoma		Control		*P
	%	N	%	N	
w1/w1	16	16	54	28	
w1/m1	77	77	46	24	0.0001
m1/m1	7	07	0	00	
Total	100.0	100	100.0	52	

*P is the value of χ^2 test.

Table 2 shows the genotype distribution between males and females in the different groups. In women, the genotype w1/m1 + m1/m1 was found at a 2-fold higher frequency in the glaucoma group (88%) than in the control group (41%) (P < 0.0001; OR = 0.1000, 95%CI = 0.0335-0.2988). In men, the w1/w1 genotype frequency was 2.75% higher in the control group (44%) than in the glaucoma group (17%) (P = 0.0259, OR = 0.2616, 95%CI = 0.0808-0.8472).

Table 2. Genotype distribution between males and females within the groups studied.

Group	Female				P*	OR	Min	Max
	Glaucoma		Control					
Genotype	%	N	%	N				
w1/w1	12	6	59	20				
w1/m1 + m1/m1	88	42	41	14	<0.0001	0.1000	0.0335	0.2988
Total	100.0	48	100.0	34				
	Male							
	Glaucoma		Control					
w1/w1	17	9	44	8				
w1/m1 + m1/m1	83	43	56	10	0.0259	0.2616	0.0808	0.8472
Total	100.0	52	100.0	18				

*P is the value of Fisher's exact test.

Table 3 shows the genotype distribution between smokers and non-smokers within the studied group. In the glaucoma group, 4 patients did not provide information about smoking. In smokers, the difference between the genotype of the *Msp*I polymorphism between groups was not statistically significant (P = 0.4643, OR = 0.0200). The frequency of the w1/w1 genotype in non-smokers in the control group (55%) was 3.44-fold higher than that in the glaucoma group (16%). This result was considered to be statistically significant (P < 0.0001, OR = 0.1501, 95%CI = 0.0674-0.3344).

Table 3. Genotype distribution among smokers and non-smokers.

Groups	Smoker				P*	OR	Min	Max
	Yes Glaucoma		Yes Control					
Genotype	%	N	%	N				
w1/w1	17	1	50	1	0.4643	0.2000	-	-
w1/m1 + m1/m1	83	5	50	1				
Total	100	6	100	2				
	No Glaucoma		No Control					
w1/w1	16	14	55	27	<0.0001	0.1501	0.0674	0.3344
w1/m1 + m1/m1	84	76	45	22				
Total	100	90	100	49				

* P is the value of Fisher's exact test.

DISCUSSION

POAG has a complex genetic basis and can be caused by a combination of genetic risk factors and environmental factors. These factors do no act in isolation. Genes that may play an important role in the pathogenesis of POAG have been intensively investigated. Different approaches are being used to study the genetics of this type of glaucoma, offering new insights into the pathogenesis of this disease (Fingert, 2011). Recent studies examining POAG in Caucasian populations from the United States revealed that disease susceptibility was linked to the *GLC1I* locus on chromosome 15q (Allingham et al., 2005; Woodroffe et al., 2006). The application of this approach to late-onset POAG is the focus of current studies in this area.

Bufalo et al. (2008) noted that the *CYP1A1*m1 polymorphism is associated with increased susceptibility to Graves' disease, which can lead to manifestations such as ophthalmopathies. Regarding the distribution of the polymorphism *CYP1A1*m1, Santos (2009) found no statistically significant difference between the groups that developed and did not develop Graves' ophthalmopathy. In our study, nearly twice as many patients in the POAG group (84%) showed the m1 polymorphic allele of the *CYP1A1* gene. This result revealed the importance of further studies examining the *CYP1A1*m1 polymorphism and POAG.

Le et al. (2003) showed that POAG development was not related to gender, which agrees with the results of Rudnicka et al. (2006). Cedrone et al. (2008) observed a high prevalence of glaucoma in female patients, which was similar to a study conducted in Dalby, Sweden (Bengtsson, 1989). The lack of consensus regarding the results had been demonstrated earlier by Mitchell et al. (1996) in Australia and by Leske et al. (1995) in Barbados. In this study, female POAG patients showed a higher prevalence of the *CYP1A1*m1 polymorphism compared to the POAG male patients.

The CYP1A1 enzyme is considered to be primarily a human extra-hepatic enzyme,

and can be induced in the lungs, lymphocytes, and placenta after exposure to polycyclic aromatic hydrocarbons, including those present in cigarette smoke (Anttila et al., 1993). The Beaver Dam Eye Study observed a minor association between smoking and POAG (Klein et al., 1993). The same result was observed by Bonovas et al. (2004), who showed that there was an increased probability that smokers and ex-smokers would develop POAG. However, Edwards et al. (2008) reported little support for the association between smoking and POAG development. Finally, Zhang et al. (2011) observed no significant association between smoking and glaucoma.

We did not observe statistically significant results for smokers in this study. In non-smoking patients, we observed that the frequency of the *CYP1A1*m1 polymorphism was 1.86-fold higher in the glaucoma group than in the control group; this result was statistically significant. We did not examine whether patients were passive smokers, how often a person smokes, or whether any subjects had stopped smoking. Exposure to cigarette smoke (passive smoking) may be a considerable risk factor for POAG (Lois et al., 2008).

Our results revealed a significant difference in the presence of the *CYP1A1*m1 polymorphism between patients with POAG and the control group. The frequency of polymorphic genotypes m1/m1 and w1/m1 in patients from the glaucoma group was approximately 1.83-fold greater than in patients in the control group.

For gender, the frequency of female patients in the glaucoma group with the polymorphic genotypes (w1/m1 and m1/m1) was 88% and the frequency of male patients was 83%. In non-smokers, the frequency of polymorphic genotypes was approximately 1.87-fold higher in the glaucoma group than in the control group. A statistically significant correlation was observed between the *CYP1A1*m1 polymorphism and POAG.

We conclude that there was a statistically significant difference in the presence of the *CYP1A1*m1 polymorphism between patients with POAG and the control group. A statistically significant correlation was observed between the *CYP1A1*m1 polymorphism and POAG.

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