GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Association between primary open angle glaucoma and genetic polymorphisms *GSTM1*/*GSTT1* in patients from Goiânia Central-West Region of Brazil

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Genet. Mol. Res. 13 (4): 8870-8875 (2014)
Received September 30, 2013
Accepted June 17, 2014
Published October 31, 2014
DOI http://dx.doi.org/10.4238/2014.October.31.2

ABSTRACT. In this study, we evaluated the genotype profile of GSTM1 and GSTT1 polymorphisms in patient carriers of primary open-angle glaucoma in the population of Goiânia, GO, Brazil. This case-control study included 100 Brazilian patients with glaucoma and 53 patients without glaucoma. Blood samples were genotyped for polymorphisms in *GST* genes using polymerase chain reaction-based methods. Polymorphism frequencies were compared using the χ^2 test and odds ratio ($\alpha = 0.05$). The *GSTM1*-present genotype was 40% in the glaucoma group and 71.6% in the control group, while the *GSTM1* null genotype was 60 and 28.3% in the same groups, respectively. The *GSTT1*-present genotype was 52% in the primary open-angle glaucoma group and 66% in the control group; the null genotype was 48% in the case group and 34% in the control group. The *GSTM1* null

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genotype was more frequent in the glaucoma group than in the control group (P = 0.0004; odds ratio = 6.7; 95% confidence interval = 2.7-20.3). The combined *GSTM1* null and *GSTT1*-present genotypes were more frequent in the primary open-angle glaucoma group compared to the control group (P = 0.02; odds ratio = 3.1; 95% confidence interval = 1.2-7.9).

Key words: Glaucoma; Glutathione S-transferase; *GSTM1*; *GSTT1*

INTRODUCTION

Primary open-angle glaucoma (POAG) is a progressive optic, chronic, and multifactorial neuropathy characterized by the loss of optic nerve fibers. The disease develops through openings in the anterior chamber of the eye, leading to abnormalities in the visual field (Saccà et al., 2005; Abu-Amero et al., 2008).

POAG is a concern for physicians, patients, and authorities who seek methods for preventing blindness from this disease. It is the second leading cause of blindness worldwide with a high incidence according to the Brazilian Consensus of Primary Open-Angle Glaucoma (Paranhos-Júnior et al., 2009). However, the early development of POAG is not fully understood. The disease is asymptomatic and is rarely a target of prevention campaigns. POAG is frequently diagnosed incidentally during ophthalmic examinations for other ocular dysfunctions (Mello and Mandía-Junior, 2005).

Several studies identified an association between POAG and glutathione S-transferase (GST) polymorphisms (Juronen et al., 2000; Yildirim et al., 2005; Ünal et al., 2007; Rocha et al., 2011). GSTs are found in a variety of ocular structures including the aqueous humor, ciliary body, and crystalline lens. The production and release of toxic metabolites may induce changes in the structures of proteins present in the aqueous humor and trabecular meshwork, leading to reduced flow and increased intraocular pressure (Izzotti et al., 2006).

Polymorphisms identified in *GSTM1* include *GSTM1*0*, *GSTM1*A*, and *GSTM1*B*. *GSTM1*0* is deleted, and homozygotes (*GSTM1* null genotype) express no protein. *GSTM1*A* and *GSTM1*B* differ by a single base, and the catalytic effectiveness of the enzymes encoded by these alleles is similar (Sprenger et al., 2000). There are 2 theta-class genes, *GSTT1* and *GSTT2*, located on chromosome 22.6. *GSTT1* is represented by 2 alleles: a functional or wild-type allele (*GSTT1*1*) and a nonfunctional or null allele (*GSTT1*0*). Studies showed that the *GSTT1*0* allele corresponds to total or partial deletion of the gene, causing a deficiency in enzymatic activity (Ünal et al., 2007).

Oxidative stress and antioxidant systems are very important in ocular tissue, which regenerates slowly, increasing the risk of toxin accumulation and resulting in tissue damage. Additionally, molecular changes in these systems contribute to the development of glaucoma, cataract, and other age-related diseases (Yildirim et al., 2005). The ocular epithelia express genes that encode for GSTs. Among the cytosolic proteins, GSTM1 and GSTT1 show the highest correlation with glaucoma (Ünal et al., 2007).

In this study, we evaluated the genotypic profile of GSTM1 and GSTT1 polymorphisms in patients who were carriers of POAG in the city of Goiânia.

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MATERIAL AND METHODS

We conducted a case-control study of 100 glaucoma patients. The control group contained 53 patients with normal eye exams that were within reference standards. The patients were treated at the Pronto Eye Clinic in Goiânia. All patients underwent ophthalmologic examination, including measurement of intraocular pressure, visual acuity test, automated perimeter test, gonioscopy, examination of the optic disc, and retinal exam. The project was approved by the National Ethics Commission in Research/National System of Information about Ethics in Research involving Human Subjects CEP/PUC-GOIÁS (FR160294), and informed consent was obtained from all patients.

Genomic DNA was extracted from peripheral blood according to the instructions in the commercial kit Illustra Blood Genomic Prep Mini Spin® (GE Healthcare, Little Chalfont, UK). Allele-specific polymerase chain reaction (PCR) of the DNA was performed in triplicate. Primer sequences for the *GSTM1* and *GSTT1* genes have been reported previously by Abdel-Rahman et al. (1996). PCR was carried out according to the protocol proposed by Frare (2011). As an internal control for the presence of human DNA and to avoid false-negatives, we used the zinc-finger gene *ZFX*/*ZFY* gene, which amplifies specific sequences on sex chromosomes (Arruda et al., 2008). One or 2 copies of this gene were classified as genotype-present, while those with homozygous deletions were classified as genotype-null.

We used the statistical analysis BioEstat® software (version 5.0; Ayres et al., 2007) to analyze the results; frequencies were determined using the chi-square test and odds ratio (OR).

RESULTS

The POAG group included 46 women and 54 men (average age of 60.5 ± 18.8 years old), while 36 women and 22 men (44.7 ± 16.4 years) were included in the control group ($P < 0.0001$).

Table 1 shows the *GSTM1* and *GSTT1* genotype frequencies for the experimental and control groups. In the glaucoma group, the *GSTM1* gene was present in 40% of patients. However, the *GSTM1* gene was detected in 72% of patients in the control group. The frequency of the *GSTM1* null gene in the glaucoma group was 2.12-fold higher than in the control group. The *GSTT1* gene was present in 52% of patients in the experimental group. In the control group, 66% of patients possessed the *GSTT1* gene, while 34% of patients were *GSTT1* null. The frequency of *GSTT1* null in the glaucoma group was 1.4-fold higher compared to the control group.

Table 1. *GSTM1* and *GSTT1* genotypic frequency in primary open-angle glaucoma (POAG) and control group.

Genotype	POAG		Control		OR (95%CI)	P
	%	N	%	N		
GSTM1						
Present	40	40	72	38	0.3 (0.1-0.5)	0.0004*
Null	60	60	28	15		
Total	100	100	100	53		
GSTT1						
Present	52	52	66	35	0.6 (0.3-1.1)	0.13
Null	48	48	34	18		
Total	100	100	100	53		

POAG = primary open-angle glaucoma. *Statistically significant value.

Table 2. *GSTM1* and *GSTT1* cluster genotypic frequency in experimental and control groups.

GSTM1	*GSTT1*	Experimental		Control		OR (95%CI)	P
		%	N	%	N		
Present	Present	23	23	47	25	1.0[1]	(Reference)
Present	Null	17	17	24	13	1.4 (0.5-3.5)	0.60
Null	Present	29	29	19	10	3.1 (1.2-7.9)	0.02*
Null	Null	31	31	10	5	6.7 (2.7-20.3)	0.0007*
Total		100	100	100	53		

*Statistically significant. [1]Reference group, individuals with genotype at low risk (*GSTM1* and *GSTT1* presence).

DISCUSSION

GSTs are found in many different ocular structures, including the aqueous humor, ciliary body, and crystalline lens (Juronen et al., 2000; Yildirim et al., 2005; Ünal et al., 2007; Rocha et al., 2011). Toxic metabolites may induce changes in the protein structures present in the aqueous humor and trabecular meshwork, leading to reduced flow and increased intraocular pressure (Izzotti et al., 2006). The eye requires an efficient, enzymatically driven detoxification system. Epidemiological studies suggest that the susceptibility to ocular pathologies are correlated with the GST system (Juronen et al., 2000; Yildirim et al., 2005).

The association *GSTM1*-null and *GSTT1*-present genotypes increase the risk of developing glaucoma by 3.1-fold. In combination, the null genotypes *GSTM1*/*GSTT1* increase the risk of developing glaucoma by 6.7-fold. Izzotti et al. (2003) analyzed an Italian population and found a higher frequency of *GSTM1*-null in glaucoma patients compared to healthy patients; this was the first association identified between GSTM1 polymorphisms and POAG. However, the *GSTT1* genotype was not statistically significant compared to the other groups, which agrees with our results.

Studies performed in the Turkish population by Yildirim et al. (2005) also agree with our results. A higher frequency of *GSTM1*-null genotype was found among glaucoma patients compared to controls (54.9 *vs* 40.9%; OR = 1.64; 95%CI = 1.10-2.59), and the frequency of *GSTT1* in both experimental and control groups was not statistically significant.

Abu-Amero et al. (2008) studied a population in Saudi Arabia and reported an association between the *GSTM1*/*GSTT1* null genotypes and POAG. Their results agree with ours and indicate that decreased GST function interferes with oxidative metabolism, leading to the optic nerve damage caused by oxidative stress; the GST polymorphism may be a risk factor for the development of POAG.

Other studies in the Brazilian population from Bahia, performed by Rocha et al. (2011), confirm our findings in the Goiás population. They reported that the *GSTM1*-null polymorphism was significantly more frequent in the POAG group than in the control group.

However, Juronen et al. (2000) analyzed the Estonia population and Ünal et al. (2007) analyzed the Turkish population and reported that the *GSTM1*-present genotype was associated with an increased risk of POAG development compared with controls. These findings differ from those observed in our study.

Studies examining autoimmunity revealed a relationship between the *GSTM1*-present genotype and glaucoma. Yang et al. (2001) showed that a GST antigen was present in 52% of patients with glaucoma and in 20% of patients in the control group (P = 0.05). Patients showed higher levels of anti-GST compared with control groups. Their results suggest that individu-

als expressing *GSTM1* are at an increased risk of developing auto-antibodies against GST and have an increased risk of glaucoma.

In contrast, Jansson et al. (2003) studied a Swedish population and detected no significant association between POAG and GST polymorphisms.

We previously studied the current group of patients and controls and found no association between the polymorphism p53 codon 72 (ARG/PRO) and POAG (Silva et al., 2009). Costa (2012) observed a statistically significant association ($P < 0.0001$) between the CYP1A1m1 polymorphism and and POAG.

Our study is one of the firsts to describe the GST polymorphism in the Central-West region of Brazil. We identified a statistically significant association between the *GSTM1*-null genotype and POAG.

Genetic studies are important for determining the mechanisms leading to the development of glaucoma (Hogewind et al., 2007). New diagnostic methods and treatments are also important. The correlation between genotype and phenotype allows the observation of different clinical manifestations as well as therapeutic responses to glaucoma. Understanding the genetic factors involved in glaucoma will contribute to earlier diagnosis and the prevention of glaucomatous damage using both conventional treatment and even gene therapy (Lopez-Martinez et al., 2007; Kwon et al., 2009; Rasool et al., 2010). In the future, it may be possible to prevent glaucoma through genetic counseling. Understanding the genetics of glaucoma is essential for decreasing disease risk. Our results may contribute to individual medical monitoring for patients with glaucoma and their families.

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