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Association of *ABCA1* (rs2472493) and *GAS7* (rs9913911) gene variants with primary open-angle glaucoma in a Brazilian population

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Purpose: Glaucoma is the world's leading cause of irreversible blindness, with primary open-angle glaucoma (POAG) being the most prevalent subtype. In recent years, there have been advances in knowledge about the genetics involved in POAG, but genetic studies in admixed populations, such as Brazilians, are still rare. This study aimed to evaluate the association of single nucleotide variants (SNV) of the *ABCA1* (rs2472493) and *GAS7* (rs9913911) genes with POAG in a sample of the Brazilian population. Furthermore, the study aimed to evaluate the relationship between these SNVs and the need for surgical intervention in glaucoma control.

Methods: A cross-sectional association study with 1,009 subjects (505 patients with POAG and 504 controls) was performed. Participants underwent a comprehensive ocular examination, including the need for surgical procedures for intraocular pressure control. Genotyping of SNVs was performed using the TaqMan genotyping assay.

Results: SNV rs9913911 of *GAS7* was found to be associated with POAG in the presence of the risk allele A ($p = 0.0004$) and the AA genotype ($p = 0.002$). There was no association between SNV rs2472493 of *ABCA1* for either the allele risk or genotypes. However, the combination of these variants showed an additive effect on the risk for POAG: *ABCA1*(GG) + *GAS7*(AA; $p = 0.02$), *ABCA1*(GG) + *GAS7*(AG; $p = 0.003$), and *ABCA1*(AG) + *GAS7*(AG; $p = 0.004$). Also, POAG patients carrying the AA genotype of the *GAS7* gene required antiglaucomatous surgery more frequently than those without the AA genotype ($p = 0.01$).

Conclusions: In a Brazilian population sample, there was an association identified between SNV rs9913911 (*GAS7*) and the risk of POAG, and an additive effect was found when *GAS7* was combined with SNV rs2472493 (*ABCA1*). There was an association between SNV rs9913911 (*GAS7*) and the risk for antiglaucomatous surgery.

Glaucoma is the world's leading cause of irreversible blindness [1,2]. About 76 million people are affected by glaucoma [2], with approximately 11.2 million progressing to bilateral blindness [1]. There are several types of glaucoma each with distinct characteristics [3], but primary open-angle glaucoma (POAG) is the most prevalent and is estimated to account for 74% of all cases [4]. Latin America has the second-highest prevalence of POAG, second only to Africa [2].

POAG is a progressive multifactorial neurodegenerative disease that affects the optic nerve and leads to the loss of retinal ganglion cells (RGCs) and their axons (nerve fibers). This degeneration is characterized by structural changes in the optic nerve head (ONH) and corresponding visual field defects [1,3,5]. Treatment is performed by reducing intraocular pressure (IOP), which is the main risk factor in determining glaucomatous damage and the only parameter that can directly act as treatment [6,7]. In some patients, IOP control is not achieved with clinical treatment; therefore, antiglaucomatous surgery is indicated.

A strong genetic component is involved in the development of POAG. Several studies have identified susceptibility loci for POAG. The first studies, performed by genetic linkage, identified genes with a monogenic pattern of inheritance, such as *MYOC*, *OPTN*, and *CYP1B1* [8–10]. Individuals with this form of inheritance usually develop the disease at an early age (<40 years) [11–14]. However, disease-causing variants in these genes are relatively rare in the population, accounting for only 5%–10% of all cases of POAG [12,15,16].

Most cases of POAG occur after the age of 40 and have a complex pattern of inheritance resulting from the interaction

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of several genes and environmental factors [13,17–21]. In this form of polygenic inheritance, each single nucleotide variant (SNV) of different genes represents a minor effect on disease pathogenesis, and when combined, they significantly affect the clinical phenotype [11–13,17,18]. In recent years, more than 120 SNVs linked to POAG have been identified through genome-wide association studies (GWAS) [22,23].

Most of these studies were conducted in Asian or Caucasian populations. Depending on the population being assessed, the same SNV may or may not have an association with POAG. Therefore, replication studies must be performed to confirm the effects of these SNVs in other regions or with other ethnicities. Among the SNVs robustly identified in multiethnic studies, the rs2472493 (*ABCA1*) and rs9913911 (*GAS7*) variants were significantly associated with POAG [22,24]. However, no individuals from South America participated in that study. Therefore, these two variants were selected to increase the odds of finding an association in our Brazilian admixed population because they could be more representative across different ethnicities. Even with the high prevalence of POAG in this region, genetic research has been scarce. To date, there has been no study of the relationship between SNVs in *ABCA1* and *GAS7* genes and POAG in Brazil. These aspects are relevant to this area of research considering the highly miscegenational Brazilian population.

The main objective of this study was to evaluate the association of the SNVs of *ABCA1* (rs2472493) and *GAS7* (rs9913911) to the risk of developing POAG in a sample of the Brazilian population. Moreover, we evaluated a) the combination of these SNVs with the risk of POAG and b) the relationship of these SNVs to the need for surgical intervention.

METHODS

A cross-sectional association case-control study was conducted at the Center for Molecular Biology and Genetic Engineering (CBMEG) and Department of Ophthalmology, Clinical Hospital, University of Campinas (UNICAMP), Campinas, São Paulo and the Regional Hospital of Divinolândia, Divinolândia, São Paulo. The study was approved by the Ethics and Research Committee of the Faculty of Medical Sciences, University of Campinas (UNICAMP). The principles of the Declaration of Helsinki were respected, and written informed consent was obtained from all participants.

Casuistic: Individuals over 40 years of age participated in the study. Participants underwent ophthalmologic examinations, and those who met the selection criteria were classified into either the case group (POAG) or the control group.

The case group was composed of patients with POAG as defined by the following criteria: a) open angle at gonioscopy (according to Foster criteria) [25]; b) IOP >21 mmHg; c) ONH with at least two criteria (cupping >0.7, cupping asymmetry >0.2, thinning of the neuroretinal rhyme, hemorrhage or notch in the ONH); or d) typical visual field defects according to Anderson's criteria [26] (Humphrey, SITA-standart 24–2 or 30–2). Surgical procedures were indicated when IOP was uncontrolled with risk or evidence of glaucoma progression.

The control group consisted of individuals over 40 years of age who had been seen at the previously mentioned ophthalmology outpatient clinics. The selected participants had ophthalmologic examinations, with results that included open angle at gonioscopy, IOP <21 mmHg, ONH cup <0.5, and no family history of glaucoma or blindness of unknown origin.

Participants who had any of the following exclusion criteria were removed from the study: secondary glaucomas, developmental glaucomas, high myopes (> −6.0 ED), fundoscopic changes compatible with age-related macular degeneration, severe diabetic retinopathies, optic neuropathies, neurodegenerative diseases, or ocular or systemic diseases that could affect a visual field examination of the optic nerve.

Genetic evaluation: All participants (cases and controls) had 5–10 ml of peripheral blood drawn into a sterile vial containing ethylene diamine tetraacetic acid (EDTA). Genomic DNA was extracted using the phenol/chloroform method.

A polymerase chain reaction was performed to amplify the region containing the studied SNVs. The genotyping of the SNVs rs9913911 of the *GAS7* gene and rs2472493 of the *ABCA1* gene for the identification of risk alleles A (*GAS7*) and G (*ABCA1*) was performed with a TaqMan assay according to the standardization protocol provided by the manufacturer (SNP Genotyping Assays TaqMan, Thermo Fisher, Waltham, MA). The genotyping results were confirmed in 10% of the samples using Sanger sequencing.

Statistical analysis: All statistical analyses were performed using R software (R Core Team, 2014). All genotypes of the case and control groups were evaluated for Hardy–Weinberg equilibrium (HWE) using the chi-square goodness-of-fit test. Analyses of gender and age were performed by Fischer's exact test and the Mann–Whitney nonparametric test, respectively. To investigate the association of the variants with POAG, a logistic regression model (stepwise) was used (Stats Package of R; R Core Team, 2014). To analyze the association between the need for antiglaucomatous surgery and the variants, the chi-square test was used. The Kruskal–Wallis

Table 1. Description of the variables sex and age among cases (505) and controls (504).

Sex/Age	Cases	Controls	p-value	OR	CI (95%)
Male	259 (51.3%)	213 (42.3%)	**0.004**	0.69	0.54–0.89
Female	246 (48.7%)	291 (57.7%)			
Age	68.1 (±11.8)	66.7 (±10.1)	**0.047**	1.01	1.00–1.02

Analyses of sex and age were performed by Fischer's exact test and the Mann–Whitney nonparametric test, respectively.

test was performed to analyze the association of the number of antiglaucomatous surgeries with the variants. Statistical significance was p <0.05.

RESULTS

The total sample size was 1,009 participants. The case group consisted of 505 subjects, 51.3% male, and the control group consisted of 504 subjects, 57.7% female (p = 0.004). The mean ages were 68.1 ± 11.8 for the case group and 66.7 ± 10.1 for the control group (p = 0.047; Table 1). Due to the differences observed in sex and age between the groups, the analyses were adjusted for these variables. Table 2 depicts the genotypic and allelic frequencies of each variant and the HWE p value. All cases and controls were in HWE (p >0.05) for the two variants studied.

Multivariate logistic regression analysis, adjusted for sex and age, was performed to analyze the association of the studied variants with the presence of POAG (Table 3). The presence of the risk allele A of the *GAS7* variant rs9913911 was found to have a significant association with POAG (p = 0.0004, OR 1.41), as well as with the AA genotype (p = 0.002, OR 1.96). For the *ABCA1* variant rs2472493, there were no significant associations with the alleles and genotypes. However, when evaluating the combination of these two variants, an additive effect was observed in the risk for POAG with the following haplotypes: *ABCA1*(GG) + *GAS7*(AA; p = 0.02), *ABCA1*(GG) + *GAS7*(AG; p = 0.003), and *ABCA1*(AG) + *GAS7*(AG; p = 0.004) (Table 4). However, in terms of age and sex, no significant associations were observed among these haplotypes.

An analysis was conducted to identify associations between the studied risk variants and antiglaucomatous surgery (Table 5). The *ABCA1* genotypes showed no association with the need for antiglaucomatous surgery, but for the *GAS7* gene, a higher number of individuals carrying the AA genotype needed antiglaucomatous surgery (p = 0.01, OR 1.90). There was no relationship between the number of antiglaucomatous surgeries and the investigated variants (Table 6).

DISCUSSION

Genetic studies related to POAG have only rarely been conducted among South American populations, including the Brazilian population, which represents approximately 49% of individuals from this region (United Nations 2020 Demographic Yearbook. 71st Issue. New York, United Nations 2021). To our knowledge, this was the first study investigating the SNVs of *ABCA1* (rs2472493) and *GAS7* (rs9913911) in a

Table 2. Descriptive analysis of genotype and allele frequencies between cases and controls.

Variant	Genotypes and Alleles	Case n (%)	P-HWE Case	Control n (%)	P-HWE Control
***ABCA1* (rs2472493)**	A/A	171 (33.9%)	0.850	187 (37.1%)	0.876
	A/G	241 (47.7%)		231 (45.8%)	
	G/G	93 (18.4%)		86 (17.1%)	
	A	583 (57.7%)		605 (60.0%)	
	G	427 (42.3%)		403 (40.0%)	
***GAS7* (rs9913911)**	A/A	264 (52.3%)	0.855	211 (41.9%)	0.980
	A/G	201 (39.8%)		231 (45.8%)	
	G/G	40 (7.9%)		62 (12.3%)	
	A	729 (72.2%)		653 (64.8%)	
	G	281 (27.8%)		355 (35.2%)	

Evaluation for Hardy–Weinberg equilibrium (HWE) was performed by the chi-square goodness-of-fit test.

Table 3. Association analysis of *ABCA1* (rs2472493) and *GAS7* (rs9913911) variants between cases (505) and controls through multivariate logistic regression.

Variant	Genotype/allele	p-value	OR	CI (95%)
***ABCA1* (rs2472493)**	GG x AA	0.36	1.18	0.82–1.69
	GA x AA	0.34	1.14	0.86–1.50
	presence G x absence G	0.35	1.09	0.89–1.34
***GAS7* (rs9913911)**	AA x GG	**0.002**	1.96	1.26–3.06
	AG x GG	0.18	1.35	0.87–2.11
	presence A x absence A	**0.0004**	1.41	1.15–1.73

OR, Odds Ratio; CI (95%), 95% Confidence Interval

Table 4. Analysis of *ABCA1* (rs2472493) and *GAS7* (rs9913911) haplotypes between cases and controls through multivariate logistic regression.

Haplotypes	p-value	OR	CI (95%)
ABCA1 **(GG)** + *GAS7* **(AA)**	**0.02**	4.11	1.18–15.43
ABCA1 **(AG)** + *GAS7* **(AA)**	0.07	2.51	0.93–6.95
ABCA1 **(GG)** + *GAS7* **(AG)**	**0.003**	6.88	1.98–25.99
ABCA1 **(AG)** + *GAS7* **(AG)**	**0.004**	4.41	1.61–12.35

OR, Odds Ratio; CI (95%), 95% Confidence Interval

sample of the Brazilian population. This was a case-control study with 1,009 participants and consisted of the replication of *GAS7* and *ABCA1* variants that were previously associated with POAG. The results are important, considering that the

Table 5. Analysis between SNVs in the *ABCA1* (rs2472493) and *GAS7* (rs9913911) genes and requirement of antiglaucomatous surgery by chi-square test.

Variant	Genotype	No Surgery n (%)	Surgery n (%)	p-value	OR	CI (95%)
***ABCA1* (rs2472493)**	AA	85 (49.7%)	86 (50.3%)	Ref.	-	-
	AG	115 (47.7%)	126 (52.3%)	0.38	1.15	0.84–1.58
	GG	36 (38.7%)	57 (61.3%)	0.05	1.47	0.99–2.19
***GAS7* (rs9913911)**	AA	120 (45.5%)	144 (54.5%)	**0.01**	1.90	1.13–3.32
	AG	95 (47.3%)	106 (52.7%)	0.20	1.42	0.83–2.50
	GG	21 (52.5%)	19 (47.5%)	Ref.	-	-

OR, Odds Ratio; CI (95%), 95% Confidence Interval.

Table 6. Analysis between SNVs of *ABCA1* (rs2472493) and *GAS7* (rs9913911) and the number of antiglaucomatous surgeries by Kruskal-Wallis test.

Variant	Genotype	Number of surgeries			p-value
		Mean	Min.	Max.	
***ABCA1* (rs2472493)**	AA	0.795	0	7	0.211
	AG	0.793	0	6	
	GG	0.892	0	3	
***GAS7* (rs9913911)**	AA	0.789	0	4	0.694
	AG	0.860	0	7	
	GG	0.725	0	3	

Statistical significance was $p < 0.05$.

Brazilian population is highly admixed and ethnically heterogeneous [28–30]. Furthermore, the analysis of these variants contributes to the creation of a genetic panel of POAG risk for Brazilians.

In this study, the frequencies of the *GAS7* rs9913911 A allele and the *ABCA1* rs2472493 G allele were 0.684 and 0.411, respectively. These frequencies are in agreement with the three largest multiethnic genomic data sets of these SNVs in the human species (TopMed, gnomAD, 1000Genome) rs9913911, rs2472493 [31,32] (Supplementary Table 1). These data indicate that due to its miscegenation, the Brazilian population could be a valuable resource for studies aimed at using admixture as a tool for mapping complex traits in humans.

SNV rs9913911 (*GAS7*) showed a significant association with POAG. Individuals carrying the A risk allele were 41% more likely to have glaucoma, and in the presence of the AA genotype, the figure was 96%, suggesting that each copy of the A allele confers an increased risk of POAG. This result is consistent with previous studies conducted in other populations. A study by Hysi and collaborators [31] examining Caucasian individuals from Australia, New Zealand, Iceland, and the USA was the first to identify the association between *GAS7* rs9913911 and POAG (p = 2.98E-13). This finding was later replicated in other studies of Caucasian (p = 2.13E-21) [32] and Japanese (p = 3.32E-04) populations [33]. A multiethnic study revealed an association between the *GAS7* variant and POAG in Caucasians (p = 6.9E-17) but not in Asians, Hispanics, and African Americans; there were no participants from South America [24]. Recently, a multiethnic meta-analysis also detected this association in Caucasians and Asians (p = 5.90E-33 and p = 1.02E-06, respectively), but not in African descendants [22] (Supplementary Table 2).

Regarding the rs2472493 variant (*ABCA1*), no significant associations of the G risk allele or GG genotype and POAG were observed, in contrast to previous studies performed in Caucasians from the USA, Europe, and Oceania [24,31,32,34] or in the Japanese population [35]. Other studies conducted with different ethnicities also found no association between rs2472493 and POAG. One was a study by Alkhatib et al. [36] with a sample of Jordanian Arabs in which there was no association of this variant with POAG (p = 0.69 for allele and p = 0.71 for genotype). Bonnemaijer and colleagues [37] conducted a multicenter study with a large number of African and African American participants, and their results also showed no significant association (p = 0.093). A multiethnic GWAS performed by Choquet and collaborators [24] reported no association of this SNV with POAG in Hispanics, Asians, or African Americans as well (p = 0.046, p = 0.90, and p = 0.018, respectively). These divergent results in different populations may be due to differences in the ancestry architecture, as well as different genetic etiologies in the development of glaucoma (Supplementary Table 3).

The effect of the combination of the risk variants of the *ABCA1* and *GAS7* genes was analyzed. This haplotype showed an additive effect on the risk of developing POAG. To date, no studies have analyzed the combination of these variants alone. The *GAS7* gene is mainly related to aqueous humor homeostasis, and variants in this gene have also been associated with increased IOP [31,38–41]. The *ABCA1* gene has also been identified in GWAS involving endophenotypes, such as IOP [31]. However, the *ABCA1* gene is associated with age-related neuroinflammatory and neurodegenerative processes, which may increase susceptibility to loss of RGCs, facilitating glaucomatous damage [42–46]. One of the possible mechanisms is *TBK1* activation, which leads to *ABCA1* ubiquitination. Its degradation reduces ANXA secretion, facilitating retinal inflammation and RGC apoptosis [47]. Furthermore, the *GAS7* and *ABCA1* genes may interact through the *ARHGEF12* gene (using ingenuity pathway analysis) and influence the risk of glaucoma by increasing IOP [48]. Both the *ABCA1* and *GAS7* genes are expressed in ocular tissues, including sclera, cornea, optic nerve, trabecular meshwork, and retina [31]. The SNV rs2472493, located upstream of *ABCA1* (Genevar database), is associated with *ABCA1* transcript levels in lymphoblastoid cell lines and may change the sequence of motifs for proteins such as FOXJ2 and SIX5. This SNV is also in high linkage disequilibrium with an SNV near *ABCA1* (rs2472494) that alters the regulatory motif for binding of the *PAX6* gene, involved in eye development. Therefore, these characteristics may suggest regulatory roles for rs2472493 and rs2472494 (near *ABCA1*) [34] in gene expression. According to the Ensembl Genome Browser, the SNV 9,913,911 (*GAS7* gene) is an intron variant located in a regulatory region, but to our knowledge, no data on how this variant might change gene expression have been reported. Therefore, when evaluating the combination of these risk variants, the increased risk of POAG may be due to the different mechanisms involved in glaucoma pathophysiology (trabecular meshwork and ONH) compacted with the hypothesis of the complex inheritance pattern of this ocular disorder. Thus, in addition to functional studies, further studies in other populations are needed to replicate these findings and to ratify this hypothesis.

The current study also evaluated whether these specific variants were associated with the need for antiglaucomatous surgery. The need for surgery may be indicative of a more severe evolution of glaucoma; however, it is important to

consider that several factors may influence the indication of a surgical procedure, such as glaucoma stage and treatment compliance, among others. These are difficult factors to control for in a study with this design, making a prospective longitudinal controlled study more suitable. In the current study, patients with POAG and the AA genotype for *GAS7* (rs9913911) had a 90% greater chance of requiring surgery for glaucoma treatment, which may indicate a more aggressive glaucoma evolution. There was no significant association regarding the number of surgeries. To date, no studies have performed this analysis.

The present study had a sample size limitation, which probably made it difficult to detect an association between rs2472493 (*ABCA1*) and POAG. This result may also indicate that the effect conferred by this variant was low in this population, which would explain the need for such a large sample size to detect an association. However, for rs9913911 (*GAS7*), it was possible to replicate previously published results and reinforce the importance of this variant with POAG. The post hoc analysis showed that the sample had a power of 80.9% to detect differences regarding the risk allele A compared to the wild-type allele G in the *GAS7* gene. Regarding the G risk allele in the *ABCA1* gene, a sample of 17,200 individuals would be required to obtain a sample power of 80% with a type I error of 5%. Given its transversal design, this study was not able to robustly address the evaluation of a combination of both alleles with regard to age of diagnosis and IOP due to a lack of information about adherence to treatment and late diagnosis of glaucoma.

The strength of this study was the careful diagnoses of the cases, performed by specialists. The controls were subjected to strict criteria, and only participants over 40 years old were included to increase the diagnostic certainty of the non-disease state. Another positive aspect was that the replication of these variants in Brazil was performed in the state of São Paulo, which is characterized by a highly admixed population [49], a factor that differentiates this study from most others that were conducted in other countries [49]. Admixed populations, such as those in Brazil, are underrepresented in genomic banks, which contain data mostly from Caucasian populations [49]. The Brazilian population is highly heterogeneous and resulted from migration events over five centuries that were accompanied by the intensive admixing of three main ancestral roots: Amerindians (the native population), Europeans, and Africans (the two main sources of migration) [29]. Brazil is a country of continental dimensions, and its genetic composition varies from region to region [29]. However, it has been demonstrated that urban populations tend to be genetically equivalent [50]. For autosomal markers, the proportion of European ancestry has been estimated at 60%–79%, with African ancestry at 10%–29% [50] and Amerindian ancestry at 7%–18% [50]. The lack of diversity in international gene banks may limit access for people of non-Caucasian ancestry to the benefits of precision medicine and more accurate testing, potentially increasing health disparities [51]. These reasons justify the need for further studies to better understand the role of genetic variants in susceptibility to POAG in the Brazilian population.

Conclusions: In conclusion, this study has shown that in this sample of the Brazilian population, the rs9913911 variant of the *GAS7* gene is significantly associated with POAG in the presence of the A allele and the AA genotype. There was no significant association between the rs2472493 variant of the *ABCA1* gene and POAG; however, when in combination with the *GAS7* risk allele, there was an additive risk effect for POAG. Additionally, there was a significant relationship between the need for antiglaucomatous surgery and the presence of the AA genotype of the rs9913911 variant of the *GAS7* gene. These findings provide further insight into the genetic profile associated with the pathogenesis of glaucoma in our population.

APPENDIX 1. FREQUENCIES OF RISK ALLELES OF THE *GAS7* (RS9913911) AND *ABCA1* (RS2472493) VARIANTS.

To access the data, click or select the words "Appendix 1."

APPENDIX 2. ASSOCIATION OF THE *GAS7* (RS9913911) VARIANT WITH POAG.

To access the data, click or select the words "Appendix 2."

APPENDIX 3. ASSOCIATION OF THE *ABCA1* (RS2472493) VARIANT WITH POAG.

To access the data, click or select the words "Appendix 3."

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