• Clinical Research •

Polymorphic variants of *ABCA1*, *PMM2*, and *ARHGEF12* genes and the risk of glaucoma in an Iranian population

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Abstract

● **AIM:** To examine whether rs2472493 and rs248032 in the *ABCA1* gene, rs3785176 in the *PMM2* gene, and rs11827818 in the *ARHGEF12* gene contribute to primary open angle glaucoma (POAG) in an Iranian population.

● **METHODS:** Totally 82 POAG patients and 172 healthy controls were enrolled. The selected gene polymorphisms were analyzed using TaqMan SNP Genotyping Assay using deoxyribonucleic acid (DNA) extracted from blood samples. Allelic and genotypic frequencies were evaluated using the Chi-square test. The association between the genotypes of single nucleotide polymorphisms (SNPs) and POAG was assessed using multiple logistic regression models. The linkage disequilibrium and haplotype block structure were assessed using the Haploview 4.2 software.

● **RESULTS:** The results showed a significant association between allele frequencies of rs2472493 in the *ABCA1* gene locus and POAG [odds ratio (OR)=1.58, 95% confidence intervals (CI)=1.04-2.39, *P*=0.031]. The rs3785176 in the *PMM2* gene was also associated with POAG in additive and over dominant genotypes. Moreover, haplotype analysis showed a significant association of two estimated haplotypes of rs2472493/rs2487032 with POAG. The AA haplotype showed a reduction in POAG risk (OR=0.41, 95%CI=0.202-0.834, *P*=0.012), while the GG haplotype was associated with the disease. In addition, this study could not discover any association between genotype and allele frequency of rs248032 in the *ABCA1* gene, and rs11827818 in *ARHGEF12* gene and POAG.

● **CONCLUSION:** rs2472493 in the *ABCA1* gene can be considered a genetic susceptibility locus for POAG. The haplotype constructed with *ABCA1* gene SNPs (rs2472493/rs2487032) is associated with POAG.

● **KEYWORDS:** *ABCA1*; *PMM2*; *ARHGEF12*; rs2472493; rs248032; rs3785176; rs11827818, Iran; primary open angle glaucoma

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INTRODUCTION

Glaucoma is one of the main causes of irreversible blindness and globally affects 7.7 million people blind or with distance vision impairment[1]. The global burden of glaucoma has increased from 1990 to 2019. However, it is expected that the age-standardized rate of disability-adjusted life years (DALY) associated with glaucoma will continue to decrease in the coming years. Lower-income regions or countries typically have higher age-standardized DALY rates[2]. During the past 30y, there has been a decrease in the average annual percent change of age-standardized prevalence and years lived with disability (YLDs) for glaucoma in most Belt and Road countries[3]. Glaucoma is a multifactorial and complex disease in which both genetic and environmental factors are involved[4-6]. High intraocular or arterial pressure, older age, low dietary intake of B vitamins, omega-3 fatty acids, nitrates, vegetables, and fruits are among the non-genetic risk factors for glaucoma[4]. Intraocular pressure (IOP) is mainly regulated by increased outflow resistance to aqueous humor at the trabecular meshwork (TM), so dysregulated

aqueous humor outflow increases IOP. Moreover, the TM cells are crucial for maintaining the normal aqueous humor outflow system, which functions as a one-way, self-cleaning, low-flow biological filter for the aqueous humor, thus helping to control the IOP[7-8]. In addition to environmental factors, that contribute to the development of glaucoma, certain biological and genetic factors may also be associated with its development. Some investigations have shown candidate genes that contribute to the onset of glaucoma such as cytochrome P450 family 1, subtype B, polypeptide 1 (*CYP1B1*); myocilin (*MYOC*); optic neurin; WD repeat domain 36; ankyrin repeat and SOCS-box containing 10, neurotrophin 4, *ABCA1*, *PMMs*, and *ARHGEF12* genes[4-6,9-12].

ATP-binding cassette (ABC) transporters are an enormous superfamily of proteins observed in all organisms. These proteins generally convey a group of complexes over cell membranes, including metabolites, drugs, vitamins, lipids, ions, and organic compounds among others[13-14]. ABC transporter A1 (ABCA1) as a member of this superfamily, mediates cholesterol efflux to lipid-free apolipoprotein AI and apolipoprotein E[13-14]. A recent investigation showed that genetic variants around the *ABCA1* gene were significantly associated with primary open angel glaucoma (POAG) through genome-wide association studies (GWAS)[9,15-16]. Furthermore, this gene is widely expressed in the TM, human retina, retinal ganglion cells and optic nerve, which play a crucial role in the development of glaucoma[17].

Phosphomannomutase 2 (*PMM2)* gene is located at 16p13.2, which produces directions for constructing an enzyme called *PMM2*[18]. Chen *et al*[9] have determined that *PMM2* is expressed in the optic nerve, TM, and other ocular tissues.

Rho guanine nucleotide exchange factor 12 is a guanine nucleotide exchange factor for the RhoA small GTPase protein that is translated by the *ARHGEF12* gene in humans, located at 11q23.3[12]. Some resources have suggested that *ARHGEF12* is a novel locus for glaucoma, but the role of *ARHGF12* is unclear[19].

Many researches have proposed the genetic association of some polymorphism with POAG[20]. Other studies in Iranians have shown that *GLIS3*, *EPDR1*, *FERMT2*, and *CHAT* genes are associated with primary angles closure glaucoma[21]. Additionally, *CYP1B1* and *LTBP2* have been recognized as genes that cause congenital glaucoma[22-23], and the *TGFB2* rs991967 polymorphism[24-25] and *MYOC* gene[26] are associated with POAG among Iranians. Since most polymorphisms have been studied in a restricted population, confirmation of their results in populations with different genetic backgrounds such as the Iranian population is required. This research aimed to examine the association of single nucleotide polymorphisms (SNPs), including rs2472493 and rs2487032 in the *ABCA1*, rs3785176 in the *PMM2*, and rs11827818 in the *ARHGEF12* genes with POAG. Additionally, this study investigated the haplotype association between two *ABCA1* gene polymorphisms (rs2472493 and rs2487032) and POAG among an Iranian population.

PARTICIPANTS AND METHODS

Ethical Approval The study was conducted in accordance with the ethical principles of Helsinki Declaration. All procedures involving participants were approved by the Ethics Committee of Shahroud University of Medical Sciences, Shahroud, Iran (930/09 and IR.SHMU.REC.1394.166). We obtained written informed consent from all participants.

Study Population This study was designed by a prevalence case-control study on the data from the 1st phase of Shahroud Eye Cohort Study (ShECS). Details of the cohort study have been described previously[27]. Briefly, the study was conducted in 2009 with the participation of 5190 people between the ages of 40-64y. These individuals were among the 6311 randomly selected individuals from Shahroud, a northeastern city in Iran. The individuals were selected using stratified cluster sampling design, which involved 300 clusters with a minimum of 20 participants each. In addition to optometry and ophthalmology examinations, blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes for genetic testing from these individuals and maintained in ultra-low freezers.

The POAG patients (cases) in this study were diagnosed according to the definition of the International Society for Geographical and Epidemiological Ophthalmology and based on the results of vertical cup to disc ratio. The characteristics of glaucoma cases have been previously reported[28]. All participants who diagnosed as POAG and had genetic lab data (82 patients), and 172 controls who selected randomly among other participants of the 1st phase of ShECS enrolled in this study. Five cases with angle-closure glaucoma were excluded from analysis. These patients were diagnosed based on the results of gonioscopy in second phase of study in 2014.

Selection of SNPs and DNA Isolation In this study, four reported functional SNPs associated with glaucoma were selected from GWAS. These SNPs are involved in the *ABCA1* (rs2472493 and rs2487032), *PMM2* (rs3785176), and *ARHGEF12* (rs11827818) genes (Table 1)[15-16,20,29-31].

Blood samples were collected in a 5 mL EDTA tube. Genomic DNA was extracted from the white blood cells using the standard phenol-chloroform DNA extraction protocol. DNA purity and concentrations were determined by the Pico-drop microliter spectrophotometer (OEM, UK). Then, DNA samples were stored for analysis at -80°C.

Genotyping Assay The genotyping of the target SNPs was conducted using the TaqMan® SNP genotyping Assay

Table 1 Detailed information of SNPs, including chromosomal position, target alleles and their roles

CHR	SNP	Position[a]	Gene	Alleles	Role
9	rs2472493	107695848	ABCA1	G>A	Intron
9	rs2487032	107703934	ABCA1	G>A	Regulatory
16	rs3785176	8896931	PMM2	A>C	Intron
11	rs11827818	120198728	ARHGEF12	A>G	Intron

CHR: Chromosome; SNP: Single nucleotide polymorphisms. [a]Position in build 37.

C_16235609_10 (rs2472493), C_11266970_10 (rs2487032), C_9088615_10 (rs3785176), and C_1968580_10 (rs11827818) from Applied Biosystems, Life Technologies Corporation, located in Forster City, CA, USA. The Bio-Rad CFX96 Real-Time polymerase chain reaction (PCR) System (Bio-Rad, Inc., Hercules, CA, USA) was used for this purpose. Each PCR reaction consisted of a total volume of 20 μL including 1×TaqMan Genotyping Master Mix (Applied Biosystems), 1×SNP Genotyping Assay Mix, and 20 ng of DNA. The real-time PCR was performed following the suggested conditions, which included an incubation at 95℃ for 10min, followed by 40 cycles of denaturation at 92℃ for 15s and annealing/extension at 60℃ for 1min. The reporter dyes VIC® and 6-carboxy-fluorescein (6-FAM) were used, and the fluorescence levels of the PCR products were evaluated at 60℃ for 1min. Additionally two no-template control (NTC) samples were included in the assay to check for contamination, following the manufacturer's protocol for placing the VIC® and FAM™-dye clusters to the origin.

Statistical Analysis The Hardy-Weinberg equilibrium was assessed using the Chi-square test. Allelic and genotypic frequencies between cases and controls in different inheritance models including dominant, recessive, and over-dominant were analyzed using Chi-square test. When considering the major allele as (M) and the minor allele as (m), the dominant model compares MM versus Mm+mm; the recessive model compares MM+Mm versus mm, and the over-dominant model compares MM+mm versus Mm[32]. The additive model assumes that each allele contributes independently to the trait. Multiple logistic regression was used to analyze the association between the genotypes of SNPs and glaucoma, reporting odds ratios (OR), 95% confidence intervals (CI) and the corresponding *P* values. For *ABCA1* SNPs, linkage disequilibrium (LD), haplotypes and their frequencies were determined using haploview 4.2 software[29]. The D' values and r^2 values were determined for the *ABCA1* SNPs. A $P<0.05$ was considered statistically significant.

RESULTS

Clinical Data The prevalence and risk factors of glaucoma in the studied population have been reported previously[28]. In the present study, 82 patients with glaucoma (case group) were examined against 172 healthy controls. The mean and standard deviation of the age of the participants in the case and control groups were 51.0±6.1 and 54.3±6.2y respectively ($P<0.001$). Among the total sample 51.6% of the participants were female and there was no significant difference in the sex proportion between the case and control groups ($P=0.249$). Additional information about the clinical and demographic characteristics of cases and controls such as age, sex, diabetes mellitus, blood pressure, smoking, and body mass index (BMI) are presented in Table 2.

Table 2 Clinical and demographic characteristics of cases and controls

Variables	Controls (*n*=172)	Cases (*n*=82)	*P*
Age, y, mean (SD)	51.0 (6.1)	54.3 (6.2)	<0.001[a]
Education, y, mean (SD)	7.7 (4.7)	6.1 (4.9)	0.011[a]
Sex			0.249[b]
Male	79 (45.9)	44 (53.7)	
Female	93 (54.1)	38 (46.3)	
Diabetes mellitus	21 (12.2)	18 (22.0)	0.044[b]
Blood pressure			0.054[b]
Normal	41 (23.8)	16 (19.5)	
Pre-hypertension	68 (39.5)	23 (28.1)	
Hypertension	63 (36.6)	43 (52.4)	
Smoking	25 (14.7)	12 (14.6)	0.988[b]
Body mass index			0.933[b]
<25	44 (25.6)	21 (25.6)	
25-29.9	74 (43.0)	37 (45.1)	
≥30	54 (31.4)	24 (29.3)	

[a]*t*-test; [b]Chi-square test.

Single Nucleotide Polymorphism Analysis The four SNPs in the studied gene were in the Hardy-Weinberg equilibrium (HWE) in all the subjects ($P>0.05$). The allele and genotype frequencies of the SNPs involved in the *ABCA1* gene are shown in Table 3. The allele frequencies of rs2472493 showed a significant overrepresentation of the G allele in cases (37.2%) compared with controls [27.3%; $P=0.031$, OR (95%CI)=1.58 (1.04-2.39)]. However, the allele frequencies of rs2487032 were not significantly different between cases and controls [$P=0.567$, OR (95%CI)=1.12 (0.75-1.68)].

The genotype frequencies of rs2472493 (risk genotype GG) were higher in the glaucoma group than in the control group ($P=0.040$). Besides, genotype distributions for rs2487032 were not significant in additive 1, additive 2, dominant and recessive models as shown in Table 3. Genotype distribution for rs3785176 was significant in additive 1 and over-dominant models. Moreover, the allele and genotyping frequencies of the rs11827818 SNPs involved in the *PMM2* and *ARHGEF12* gene showed no significant representation in cases compared with controls and were not associated with glaucoma risk in inheritance models (Table 4).

Table 3 Models for analysis of the association between *ABCA1* gene polymorphism and glaucoma

SNP	Model		Sample size (%)		Multiple logistic regression model[a]	
			Glaucoma cases	Healthy controls	Odds ratio (95%CI)	*P*
rs2472493	Allele	G *vs* A	61 (37.2); 103 (62.8)	94 (27.3); 250 (72.7)	1.58 (1.04-2.39)	0.031
	Additive 1	AG *vs* A	33 (48.5); 35 (51.5)	60 (38.7); 95 (61.3)	1.41 (0.77-2.56)	0.263
	Additive 2	GG *vs* AA	14 (28.6); 35 (71.4)	17 (15.2); 95 (84.8)	2.50 (1.04-5.96)	0.040
	Dominant	GG+AG *vs* AA	47 (57.3); 35 (42.7)	77 (44.8); 95 (55.2)	1.60 (0.92-2.79)	0.098
	Recessive	GG *vs* G+AA	14 (17.1); 68 (82.9)	17 (9.9); 155 (90.1)	2.07 (0.92-4.65)	0.077
	Over-dominant	AG *vs* A+GG	33 (40.2); 49 (59.8)	60 (34.9); 112 (65.1)	1.16 (0.66-2.06)	0.607
rs2487032	Allele	G *vs* A	103 (62.8); 61 (37.2)	211 (61.3); 133 (38.7)	1.12 (0.75-1.68)	0.567
	Additive 1	GA *vs* GG	37 (75.5); 12 (24.5)	71 (69.6); 31 (30.4)	1.26 (0.56-2.84)	0.586
	Additive 2	AA *vs* GG	33 (73.3); 12 (26.7)	70 (69.3); 31 (30.7)	1.26 (0.56-2.83)	0.572
	Dominant	AA + GA *vs* GG	70 (85.4); 12 (14.6)	141 (82.0); 31 (18.0)	1.28 (0.60-2.70)	0.523
	Recessive	AA *vs* GA+TT	33 (40.2); 49 (59.8)	71 (41.3); 101 (58.7)	0.91 (0.52-1.60)	0.751
	Over-dominant	GA *vs* AA+GG	37 (45.1); 45 (54.9)	74 (42.0); 102 (58.0)	1.05 (0.60-1.83)	0.860

[a]Adjusted for age, sex, and education of participants. CI: Confidence Interval.

Table 4 Models for analysis of the association between rs3785176 and rs11827818 polymorphism and glaucoma

SNP	Model		Sample size (%)		Multiple logistic regression model[a]	
			Glaucoma cases	Healthy controls	Odds ratio (95%CI)	*P*
rs3785176	Allele	C *vs* A	26 (15.9); 138 (84.1)	67 (19.5); 277 (80.5)	0.75 (0.45-1.26)	0.278
	Additive 1	AC *vs* AA	18 (23.1); 60 (76.9)	57 (34.1); 110 (65.9)	0.52 (0.27-0.996)	0.048
	Additive2	CC *vs* AA	4 (6.2); 60 (93.8)	5 (4.3); 110 (95.7)	1.76 (0.42-7.44)	0.444
	Dominant	CC+CA *vs* AA	22 (26.8); 60 (73.2)	62 (36.1); 110 (63.9)	0.60 (0.32-1.10)	0.098
	Recessive	CC *vs* CA+AA	4 (4.9); 78 (95.1)	5 (2.9); 167 (97.1)	2.10 (0.50-8.76)	0.307
	Over-dominant	CA *vs* CC+AA	18 (21.9); 64 (78.1)	57 (33.1); 115 (66.9)	0.50 (0.26-0.96)	0.036
rs11827818	Allele	A *vs* G	23 (14.0); 141 (86.0)	49 (14.2); 295 (85.8)	1.02 (0.59-1.79)	0.935
	Additive 1	GA *vs* GG	19 (90.5); 2 (9.5)	39 (88.6); 5 (11.4)	1.24 (0.20-7.69)	0.820
	Additive2	AA *vs* GG	61 (96.8); 2 (3.2)	128 (96.2); 5 (3.8)	1.12 (0.18-6.94)	0.901
	Dominant	AA+GA *vs* AA	80 (97.6); 2 (2.4)	167 (97.1); 5 (2.9)	0.98 (0.17-5.67)	0.981
	Recessive	AA *vs* GA+AA	61 (74.4); 21 (25.6)	128 (74.4); 44 (25.6)	0.97 (0.52-1.83)	0.934
	Over-dominant	GA *vs* GG+AA	19 (23.2); 63 (76.8)	39 (22.7); 133 (77.3)	1.03 (0.54-1.97)	0.939

[a]Adjusted for age, sex, and education of participants. CI: Confidence interval.

The analysis of LD and haplotype block structure showed the SNPs rs2472493 and rs2487032 were in strong LD in the whole population (D'=0.79, r^2=0.44). The extent of LD was different between the glaucoma and control groups, confirming the region between SNPs rs2472493 and rs2487032 as a locus of putative association (Figure 1; Table 5).

Table 5 Haplotype frequencies of rs2472493 and rs2487032 in glaucoma, Shahroud, Iran, 2009

Haplotype	Controls	Cases	*P*[a]
A-A	0.140	0.066	0.014
A-G	0.246	0.306	0.153
G-G	0.027	0.066	0.035
G-A	0.587	0.562	0.605

[a]Chi-square test.

DISCUSSION

To the best of our knowledge, this is the first population-based study exploring an association between the *ABCA1*, *PMM2*, and *ARHGEF12* gene polymorphism and POAG risk in an Iranian population. In this study, we found a significant association between allele frequencies of rs2472493 (G as the risk allele) in the *ABCA1* gene locus and POAG in the Iranian population (*P*=0.031, OR=1.58). However, the genotype frequencies of rs2472493 SNP displayed no significant difference between cases and controls and were not associated with POAG risk in inheritance models. The AA haplotype was associated with a decreased risk of POAG, while the GG haplotype was linked to an increased risk in the studied population.

The *ABCA1* gene belongs to a member of the ABC transporter family, which encodes a membrane-associated protein[33]. This protein complex transports multiple molecules across intracellular and extracellular membranes. Mutation in

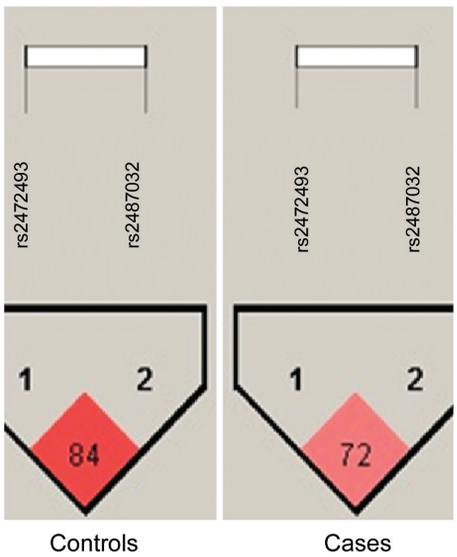

Figure 1 Linkage disequilibrium patterns in both controls and cases The extent of LD is lower in case chromosomes than in control chromosomes. Four predicted haplotypes derived from these two SNPs and their frequencies are shown in Table 5. Haplotype analysis showed a significant association of two estimated haplotypes of rs2472493/rs2487032 with glaucoma. The AA haplotype showed a reduction in POAG risk (OR=0.41, 95%CI=0.202-0.834, *P*=0.012), while the GG haplotype was associated with an elevated risk in POAG (OR=2.727, 95%CI=1.056-7.045, *P*=0.032). However, the other two haplotypes were not related to the POAG risk. LD: Linkage disequilibrium; SNPs: Single nucleotide polymorphisms; POAG: Primary open angle glaucoma; OR: Odds ratio; CI: Confidence interval.

the *ABCA1* gene has been related to familial high-density lipoprotein deficiency and Tangier disease[13]. Chen *et al*[9], Gharahkhani *et al*[15], and Luo *et al*[29] have revealed that in glaucoma pathogenesis, *ABCA1* is an important nominee gene because its mRNA and protein are expressed in human TM, ciliary body, iris, optic nerve, cornea, and retina; principally in the ganglion cell layer. They have affirmed that the expression of *ABCA1* protein is related to glaucoma and non-glaucoma eyes. Besides, Hysi *et al*[16] reported that the IOP-related variant rs2472493 is connected with *ABCA1* transcript levels in lymphoblastoid cell lines. Additionally, *ABCA1* may regulate neuroinflammation and neurodegeneration in the mouse brain, which could plausibly play a role in retinal and glaucoma pathogenesis[15-16].

Briefly, Gharahkhani *et al*[15] have performed a GWAS in an Australian discovery cohort involving 1155 advanced POAG patients and 1992 healthy controls. Subsequent to a Meta-analysis of all cohorts, they issued three novel loci associated with the development of glaucoma. One of these loci rs2472493[G] is located upstream of *ABCA1* on chromosome 9. They founded that SNP rs2472493 in the *ABCA1* gene is considerably associated with POAG in Australian cohorts, and it is a novel risk locus that might be included in developing POAG (P=2.1×10^{-19}, OR=1.31). These findings were repeated in other independent GWAS in the China and GERA and African populations[30-31,34]. Furthermore, Hysi *et al*[16] summarized the results of a GWAS Meta-analysis of 18 population cohorts from the international glaucoma Genetics Consortium (IGGC), including 35 296 multi-ethnic participants for IOP. They concluded the genetic relationship between rs2472493 loci and glaucoma. However, this association was not significant in a Saudi population after adjusting for age and sex[35-36], and also in a Brazilian population[37], indicating the diversity of genetic risk factors of POAG in different races and ethnicities. Different susceptibilities of Asians compared to Caucasians for other polymorphisms associated with glaucoma have also been reported[38].

Chen *et al*[9] have examined 1007 glaucoma patients with high-pressure glaucoma, and 1009 controls form southern China. They found a significant association at rs2487032 representing *ABCA1* (P=2.79×10^{-19}), and rs3785176, representing *PMM2* (P=5.77×10^{-10}). They proposed that *ABCA1* and *PMM2* are expressed in the optic nerve, TM, and other ocular tissues. Another study in Chinese subjects also confirmed the association of rs2487032 with POAG[39]. However, our study has illustrated that the rs2487032 SNP does not show any significant difference in cases compared to controls, and it is not associated with an increased risk of POAG. On the other hand, we did find that the rs3785176 SNP is associated with an increased risk of POAG in both additive and over-dominant models, which imply that this SNP is not a major factor influencing the POAG. This discrepancy in results may be due to variations in genetic risk factors among different populations, differences in study sample sizes, and variations in study methods. Therefore, it is important to prioritize more rigorous studies conducted in various countries.

Additionally, Wang *et al*[34] have investigated the connection between the rs2487032 SNP and glaucoma patients, which included 500 glaucoma cases and 720 controls. They suggested that the allele frequencies of rs2487032 were not significantly different between patients and controls (P=0.5304, OR=0.942). Moreover, they recommended that the genotype frequencies of rs2487032 SNPs presented no significant difference in cases from controls (P=0.471, OR=1.126). In contrast to the findings of Chen *et al*[9] and Wu *et al*[39] and consistent with the findings of Wang *et al*[34] no significant allelic/genotype effect of the rs2487032 SNP in POAG was observed in our study.

The GWAS, conducted by Gharahkhani *et al*[15] additionally, showed a significant association at the rs11827818[G] close to the *ARHGEF12* gene (P=9.2×10^{-9}, OR=1.52) on chromosome 11. However, the findings of the current study do not support Gharahkhani *et al*'s[15] results and we showed that rs11827818

is not a risk locus that might be involved in developing POAG.

Besides, the results of our study showed a significant linkage disequilibrium between rs2472493 and rs2487032, and we observed that the haplotype AA conferred a reduced risk of glaucoma, whereas the haplotype GG showed an increased risk of glaucoma. In contrast to our study, a previous study did not report a significant relationship between *ABCA1* haplotypes (rs2472493 and rs2487032) and glaucoma risk[17,34]. The discrepancy in these results might be attributed to different populations, sample sizes and haplotype blocks.

It is plausible that a number of limitations may have influenced the results obtained. Although the total sample size in the cohort study was considerable, the number of glaucoma patients was limited and only a restricted number of polymorphisms were examined. We have low statistical power in some associations and the negative results should be interpreted with caution. In addition, interaction with other genetic or environmental factors was not examined in the current study. It is now clear that environmental factors such as air pollution, physical activity, and diet are associated with glaucoma[40]. Adjusting the multiple regression models to include these covariates may alter the results. Further studies with larger sample sizes and multi-factor analysis in different ethnic groups are required.

In conclusion, the current study identified rs2472493 in the *ABCA1* gene as a genetic susceptibility locus for glaucoma in an Iranian population. Besides, the haplotype constructed with *ABCA1* gene SNPs (rs2472493/rs2487032) was associated with glaucoma. The rs3785176 polymorphism was also associated with POAG in additive and over dominant genotypes. These results, in conjunction with previous research, place the *ABCA1* gene as an important genetic risk marker for POAG. The results of such studies can lead to the identification of prognostic genetic factors that can eventually be utilized to distinguish and screen susceptible individuals before a reduced visual field and blindness. However, further studies are required to establish the role of the *ABCA1* gene in glaucoma prognosis and to confirm the role of the *ABCA1* gene in the pathogenesis of glaucoma.

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