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Significant Association and Increased Risk of Primary Open Angle Glaucoma with *TGFB2* Rs991967 Gene Polymorphism in North Eastern Iranian Patients

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Abstract

Background: Glaucoma is one of the main causes of irreversible blindness. The most common type of glaucoma is primary open angle glaucoma (POAG). TGF-β2, the main TGF-β isoform in the eye, is critical for extracellular matrix production and angiogenesis. Genetic studies have shown that TGF-β2 gene (*TGFB2*) polymorphisms affect its expression in the eye. The aim of this study was to investigate the presence of the *TGFB2* rs991967 polymorphism in POAG, and the effect of this polymorphism on clinical characteristics in POAG patients.

Methods: This case-control study was conducted on 112 control participants and 112 POAG patients referred to Khatam-Al-Anbia Eye Hospital, Mashhad, Iran. The *TGFB2* rs991967 polymorphism was genotyped by the PCR-restriction fragment length polymorphism (PCR-RFLP) method. The genotyping results and clinical findings were analyzed using SPSS version 16.

Results: The most common genotype was AA, observed in 54.5% of the patients ($P < 0.0001$, OR 12.2, CI 95% for OR: 5.25 to 28.31). Moreover, the highest and lowest frequencies of the mutant A allele were seen in the patient and control groups with percentages of 73 and 40%, respectively. This difference was significant ($P < 0.0001$, OR: 3.9, CI 95% for OR: 2.6 to 5.9). No significant association was seen between the frequencies of the *TGFB2* rs991967 polymorphism genotypes and clinical characteristics in POAG patients.

Conclusions: The *TGFB2* rs991967 polymorphism has a direct and significant association with POAG and significantly increases the risk of developing POAG.

Keywords: PCR, Polymorphism, Primary Open Angle Glaucoma, TGF-beta2.

Introduction

Glaucoma is one of the leading causes of irreversible blindness. Optic neuropathy is a major characteristic of glaucoma. In this case, enlargement of the optic nerve cup and disruption of the visual field is the main clinical findings. The main causes of this condition are age and increased intraocular pressure (IOP) in the eyes. The most common type of glaucoma is primary open angle glaucoma (POAG). This type of glaucoma occurs in 1-2% of the population over age 40. The anterior chamber angle and trabecular meshwork are normal in POAG patients (1-5).

Transforming growth factor beta (TGF-β) is growth factor that plays crucial roles in cellular functions, including induction of extracellular matrix production and angiogenesis. TGF-β

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comprises five isoforms, referred to as TGF-β1 through TGF-β5. Only three of these isoforms, TGFs-β1, β2, and β3 are found in mammals. TGF-β2, the main isoform in the eye (6, 7), has been identified in the eye aqueous, vitreous, and tears (8-10). TGF-β2 is first synthesized as an inactive polypeptide of about 25 kDa. The activation process of this molecule is not exactly specified, but it seems to have roles in regulating its function (6, 11).

Chondroitin sulfate is a major component of the extracellular matrix of the trabecular meshwork, which affects aqueous humor outflow from the eye. Studies have shown that TGF-β2 plays a basic role in the production of proteoglycans, including chondroitin sulfate, in the eye. Proteoglycan production in the eye can reduce aqueous humor outflow facility, thus increasing IOP, the main risk factor for glaucoma (12, 13). Studies have shown that TGF-β2 levels are significantly greater in patient's aqueous humor with POAG than in healthy individuals (14, 15).

Genetic studies have shown that the TGF-β2 gene (*TGFB2*), located on chromosome 1, has several polymorphisms that affect its expression. Of these polymorphisms, *TGFB2* rs991967 affects *TGFB2* expression in the eye (16).

Due to the importance of POAG recognition, the presence of the *TGFB2* rs991967 polymorphism in POAG, and a potential effect of the polymorphism on the clinical characteristics of POAG, this study was conducted. Given the high frequency of glaucoma in populations and the importance of early diagnosis, identifying major risk factors associated with glaucoma are important.

Materials and methods

Study design and subjects

This case-control study included 112 healthy controls and 112 POAG patients referred to the glaucoma clinic of Khatam-Al-Anbia Eye Hospital, Mashhad, Iran. POAG diagnoses in the patients and recruiting of control participants were performed by an ophthalmology specialist**.** After obtaining written consent from all study participants, demographic information was collected. The inclusion criteria for control participants were age over 40 years, referring to other clinics of Khatam-Al-Anbia Eye Hospital, and negative diagnosis and no family history of glaucoma. The inclusion criteria for diagnosis of POAG were increased cup-to-disc ratio (CDR) with a normal appearing open angle in gonioscopy, increased IOP, or normal IOP when using antiglaucoma drugs, and abnormal perimetry changes. Exclusion criteria were histories of ocular trauma, uveitis, abnormal gonioscopy, eye surgery, or laser iridectomy. Slit lamp and fundus examinations were performed on all participants and visual acuity (VA), CDR, and IOP (by Goldman tonometry) were measured and recorded.

TGFB2 rs991967 polymorphism genotyping

Blood was collected from each participant and genomic DNA was extracted by the salting out method as previously described (17). The *TGFB2* rs991967 polymorphism was genotyped by the PCR-restriction fragment length polymorphism (PCR-RFLP) method.

The PCR-RFLP was performed using the primers and HpyCH4III restriction enzyme shown in Table 1. The PCR amplification program was as follows: initial denaturation at 95 °C for 4 min followed by 35 cycles of denaturation at 94 °C for 45 sec, annealing at 55 °C for 30 sec, extension at 72°C for 45 sec, and a final extension at 72 °C for 5 min.

To genotype *TGFB2* rs991967**,** the amplified sequences were digested as follows: 10 µl of PCR product were incubated with 0.25-0.5 units of HpyCH4III for 24 hr at 37 °C and the digested products were separated by 2% agarose gel electrophoresis and visualized using a UV light.

Table 1. Primer sequences and restriction enzyme for *TGFB2* rs991967 polymorphism genotyping

Primers	Restriction enzymes	PCR product	DNA fragment size
Forward: 5′-TGACCGAGAAAGTCTGCATT-3′ Reverse: 5′AAGGTCTGAAGTTTGACCAGTACA-3′	HpyCH4III	239 bp	A: 239 bp C: 100+139 bp

Statistical analysis

Hardy-Weinberg equilibrium for genotype distribution analyses, descriptive statistics, independent t-test, one-way ANOVA, chi-square, Fisher's exact test, and logistic regression test, and alternative nonparametric tests of the statistical tests, if applicable, were used to analyze the results. $P < 0.05$ was considered significant.

Results

One hundred twelve POAG patients and 112 healthy controls were included in the study. No gender differences were found between the groups ($P = 0.44$); however, in both groups, the number of males was greater than that of females. Also, no significant age differences were found between the groups (Table 2).

Table 2. Demographic characteristics of the POAG and control group's participants.

	Number of participants	Sex (%)	Age (years) Mean ± SD
POAG patients	112	Male: 69 (61.6%) Female: 43 (38.4%)	57.8±13.7
Controls	112	Male: 71 (63.4%) Female: 41(36.6%)	58.9±13.5
P value	--	0.44*[1]	0.54*[2]

*1: Chi Square Test, *2: Independent T test

The *TGFB2* rs991967 genotype distributions were in Hardy-Weinberg (HW) equilibrium in both case (HW $P = 0.47$) and control (HW $P = 0.12$) groups. Significant differences in genotype frequencies were seen between the patient and control groups for the codominant, dominant, and recessive inheritance models.

In the codominant model, the AA genotype was the most common in the patient group at 54.5%, while the CC genotype was the most common in the control group at 39.3% ($P < 0.0001$, odds ratio (OR) for AA genotype: 12.2, confidence interval (CI) 95% for OR: 5.25 to 28.31). In the dominant and recessive inheritance models, in the patient group, the AA genotypes were 91.1 and 54.5%, respectively which were both significantly greater than in the control group ($P < 0.0001$). Finally, the mutant A allele was the most common allele in the patient group at 73%, and the least common in the control group at 40%. This difference was statistically significant ($P < 0.0001$, OR: 3.9, CI 95% for OR: 2.6 to 5.9) (Table 3).

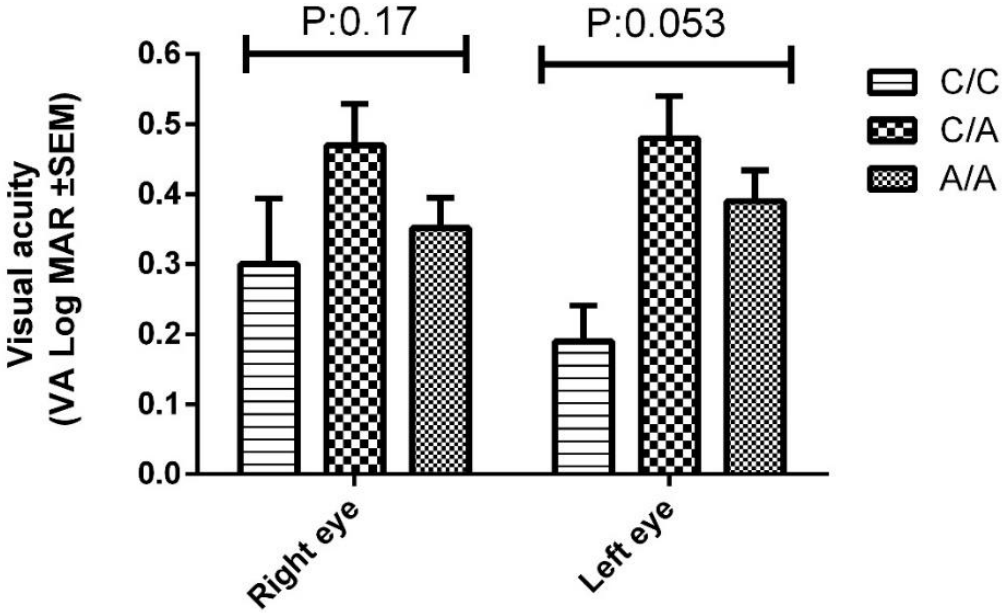

Fig. 1. Visual acuities in the right and left eyes of POAG patients with the C/C, C/A, and A/A *TGFB2* rs991967 genotypes.

Table 3. Genotype and allele frequencies of *TGFB2* rs991967 polymorphism in POAG and control groups.

TGFB2 (rs991967)		Controls n = 112	POAG patients n=112	P value	OR (CI 95%)*[3]
Codominant					
	C/C	44 (39.3%)	10 (8.9%)	<0.0001*[1]	1.0 (reference)
	C/A	46 (41.1%)	41 (36.6%)		3.92 (1.75 to 8.77)
	A/A	22 (19.6%)	61 (54.5%)		12.2 (5.25 to 28.31)
Hardy Weinberg P value		0.12	0.47		
Dominant	C/C	44 (39.3%)	10 (8.9%)	< 0.0001*[1]	1.0 (reference)
	A/A-C/A	68 (60.7%)	102 (91.1%)		6.6 (3.1 to 14)
Recessive					
	C/A-C/C	90 (80.4%)	51 (45.5%)	< 0.0001*[2]	1.0 (reference)
	A/A	22 (19.6%)	61 (54.5%)		4.8 (2.6 to 8.8)
Overdominant					
	A/A-C/C	66 (58.9%)	71 (63.4%)	0.58*[2]	1.0 (reference)
	C/A	46 (41.1%)	41 (36.6%)		0.82 (0.48 to 1.4)
Alleles					
	C	134 (60%)	61 (27%)	< 0.0001*[2]	1.0 (reference)
	A	90 (40%)	163 (73%)		3.9 (2.6 to 5.9)

*1 Chi square test, *2: Fisher's exact test, *3: Logistic regression test

Clinical characteristics and the *TGFB2* rs991967 polymorphisms are shown in Figs. 1, 2, and 3. The VA of POAG patients was least in the left eye of those with the CC genotype with an average of 0.48 ± 0.06 VA log MAR ± SEM, and greatest in the left eye of those with the CA genotype with an average of 0.19 ± 0.05 VA log MAR ± SEM. No significant differences in VA were seen in either eye between subjects with different *TGFB2* genotypes (P = 0.17 and 0.053 in in the right and left eyes, respectively) (Log Mar: reverse logarithm of VA) (Fig. 1).

The greatest IOP was seen in the right eye of patients with the AA genotype with an average of 25.43 ± 1.69 mmHg ± SEM and the least in the left eye of patients with the CA genotype with an average of 18.39 ± 1 mmHg ± SEM; however, these differences were not significant (P = 0.14 and 0.16 for the right and left eyes, respectively) (Fig. 2).

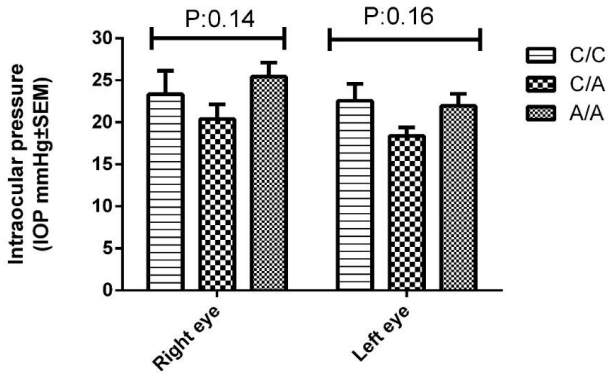

Fig. 2. Intraocular pressures in the right and left eyes of POAG patients with the C/C, C/A, and A/A *TGFB2* rs991967 genotypes.

The greatest CDR was seen in the right eye of AA genotype patients with an average of 0.77 ± 0.03 CDR ± SEM, and the least in the right eye of the CA genotype patients with an average of 0.64 ± 0.04 CDR ± SEM. This difference was not significant (P = 0.61 and 0.82 for the right and left eyes, respectively) (Fig. 3).

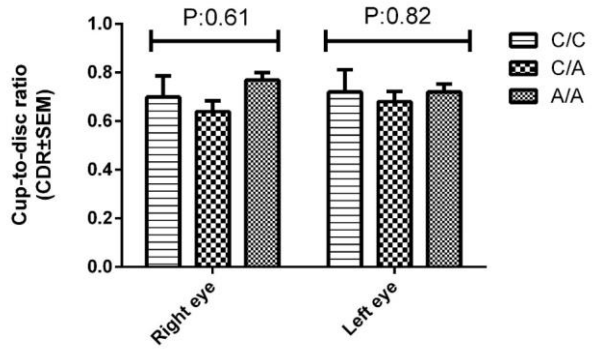

Fig. 3. Cup-to-disc ratios in the right and left eyes of POAG patients with the C/C, C/A, and A/A *TGFB2* rs991967 genotypes.

Discussion

In this study, the potential association between the *TGFB2* rs991967 polymorphism and POAG and clinical findings were investigated. We found that the *TGFB2* rs991967 polymorphisms were significantly associated with POAG.

The TGF- β1, β2, and β3 isoforms are found in the eye. Of these, TGF-β2 is the most abundant and is usually found in the aqueous humor. Several studies have reported the role of TGF-β isoforms in the development of myopia and POAG (6, 7). In 2008, Jobling et al. reported that various TGF-β isoforms contributed to the pathogenesis of myopia and TGF-β2 was elevated in untreated myopia patients and reduced after myopia treatment (18).

Jia et al., in 2014, reported that TGF-β2 levels in aqueous humor were directly associated with myopia. Their results suggested that TGF-β2 can cause axial elongation, which ultimately leads to ocular complications such as myopia (19).

Following a meta-analysis of 12 studies related to TGF-β2 and POAG, Agarawal et al. concluded that the active and total form of ocular TGF-β2 was increased dramatically in POAG patients' eyes (20). Further studies were then conducted to investigate the possible presence of gene polymorphisms involved in POAG pathogenesis. Polymorphisms, depending on their function, can reduce or induce the synthesis of factors that contribute to disease pathogenesis. In 2009, Lin et al. showed that the *TGFB2* rs7550232 polymorphism is associated with the development of myopia (16).

Present study results indicate that individuals with the *TGFB2* rs991967 polymorphism have a higher risk of developing POAG than healthy controls; however, we found no significant association between the *TGFB2* rs991967 polymorphism and clinical features of the disease. Based on these results, it is likely that the *TGFB2* polymorphisms contribute to disease occurrence, but not severity. Various theories have been proposed to explain how *TGFB2* polymorphisms could play a role in the development of POAG. The main proposed roles of TGF-β2 associated with POAG and myopia are inhibition of vascular endothelial cell production and stimulation of α-smooth muscle actin, fibronectin matrix protein, elastin, and proteoglycan syntheses, which can cause ocular complications, including POAG and myopia (18, 20-22).

To more precisely determine the association between gene polymorphisms and disease susceptibility, our PCR-RFLP results should be confirmed by DNA sequencing. Moreover, the small sample size and incomplete demographic data of case and control participants were limitations of our study. Also, the use of sensitive measures of disease severity such as the visual field test and retinal nerve fiber layer (RNFL) thickness would increase the power of the study to detect a possible correlation between disease severity and genetic variations.

Previously, we reported that *TGFB1 -509C>T* gene polymorphism increase the risk of POAG in Iranian population (23). According to this study, the *TGFB2* rs991967 polymorphism has a direct and significant association with POAG; however, no significant association was found between the *TGFB2* rs991967 polymorphism and clinical characteristics in POAG patients. We recommend that other polymorphisms of genes involved in glaucoma, as well as the quantitative analysis of these gene products and their association with ocular diseases, be studied.

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