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Genetic Diagnosis of Glaucoma

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Authors' contributions

This work was carried out in collaboration between both authors. Author HM designed the study, collected clinical data, performed the statistical analysis, wrote the protocol, managed the literature searches and wrote the first draft of the manuscript. Author MSH managed the analysis of the study and PCR method. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Purpose: Glaucoma, the most prevalent cause of irreversible blindness across the world, is progressive optic nerve degeneration and affection (neuropathy) caused by a mixture of both genetic and environmental factors [1]. The extracellular matrix (ECM) structure of the trabecular Meshwork TM has a major role in intraocular pressure IOP control. Transforming growth factor beta (TGF- β) is a growth factor that plays major roles in cellular functions, including encouraging extracellular matrix synthesis and vascular angiogenesis. TGF β 2 treatment of TM cells alters ECM components [8] and induces ECM bonds.

Aim of the Study: To study the relationship between family history and glaucoma according to genotype and genetic polymorphism.

Methods: Blood collection and DNA extraction Genotyping: TGFB2 Rs99196 genotyping was done using TaqMan SNP genotyping Assay (ID C__8853564_10). StepOne real time PCR system (Applied Biosystem, Ca, USA) was used for amplification.

Statistical Analysis: The sample size of the study group was calculated using a program at (www.openepi.com/SampleSize/SSCC.htm).

Results: Important genotype differences frequencies were detected between the positive family history and negative family history groups for the codominant, dominant, recessive and overdominant inheritance models.

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Conclusion: This study recommends that other polymorphisms of genes associated with glaucoma and the analysis of these gene products and their relationship with disease risk factors should be more studied.

Keywords: Glaucoma; diagnosis; genotype; genetic polymorphism.

1. INTRODUCTION

Glaucoma, the most prevalent cause of non-curable blindness across the world, is a progressive optic neuropathy (optic nerve affection) caused by a combination of both genetic and environmental factors [1].

It is a heterogeneous group of diseases characterized by a loss of retinal ganglion cells (RGCs) and their axons in the optic nerve, leading to different degrees of visual field defects. Primary open-angle glaucoma (POAG) is the most frequent form of glaucoma [2], and increased intraocular pressure (IOP) is a major risk factor, although approximately one-third of POAG patients have IOP levels within the normal range [3]. In addition to high IOP, the major risk factors for POAG include old age, myopia, ethnicity, and positive family history [4].

The extracellular matrix (ECM) components of trabecular meshwork TM have a major role in IOP regulation [5]. The ability of the TM to respond to dynamic changes in IOP in a homeostatic state depends mainly on the ECM remodeling capabilities of the TM [6]. Increased protein deposition of ECM in the TM increases aqueous humor AH outflow resistance, and elevated IOP is all linked with primary open-angle glaucoma (POAG) [7].

Transforming growth factor beta (TGF- β) is a growth factor that plays a major role in cellular functions, including extracellular matrix production and vascular angiogenesis. TGF β 2 treatment of TM cells alters ECM composition [8] and induces ECM cross-linking [9].

It is well recognized that TGF- β 2 levels are significantly elevated in patient's aqueous humor of patients with POAG. [10]. Genetic studies clarified that the TGF- β 2 gene (TGFB2), located on chromosome number 1, has many polymorphisms that affect its expression and function. Of these polymorphisms, TGFB2rs991967 affects TGFB2expression in the eye [11].

The high prevalence of glaucoma in populations and the importance of early detection and diagnosis, identifying the genetic factors influencing an individual's susceptibility to POAG are important.

1.1 Aim of the Study

To study the relationship between family history and glaucoma according to genotype and genetic polymorphism.

2. METHODS

The study was approved by the Sohag Faculty of Medicine Ethics Committee, and informed written consent was obtained from all participants. The study was carried out on 200 participants (100 with and 100 without a family history of glaucoma); the glaucoma was diagnosed by an ophthalmologist using fundus examination and optical coherence tomography recruited from the Department of Ophthalmology, Faculty of Medicine, Sohag University from the period of March 2019 to June 2019. The inclusion criteria participant's age was over 40 years. Exclusion criteria were histories of ocular trauma, uveitis, cataract, retinal disease, abnormal gonioscopic, and eye surgery.

2.1 Blood Collection and DNA Extraction

About 5 mL venous blood was collected from each participant in ethylene-diamine-tetraacetic acid (EDTA) tubes under aseptic conditions and centrifuged at 3000 \times g for 15 min. The mononuclear cell layer was immediately used for DNA extraction. DNA extraction was done using a spin column based Blood-Animal-Plant DNA Preparation Kit supplied by Applied Biosystems, according to enclosed instructions.

2.2 Genotyping

TGFB2 Rs99196 genotyping was done using the TaqMan SNP genotyping assay (ID C__8853564_10). StepOne real-time PCR system (Applied Biosystem, CA, USA) was used for amplification. Amplification was done using a 25 μ L reaction volume containing 12.5 μ L

TaqMan® Genotyping Master Mix, 1.25 µL specific TaqMan® SNP genotyping assay (containing sequence-specific forward and reverse primers to amplify the polymorphic sequence of interest and two TaqMan® MGB probes; one probe labeled with VIC® dye to detect the allele 1 sequence and another probe labeled with FAM™ dye to detect the allele 2 sequence) and 5 µl (20 ng) of genomic DNA. The reaction mixture was held at 95 °C for 10 min for AmpliTaq Gold enzyme activation, followed by 40 amplification cycles. Each cycle consisted of denaturation at 95 °C for 15 s, primer annealing, and extension at 60 °C for 60 s. The study data were analyzed using TaqMan® Genotyper™ Software.

The codominant, dominant, recessive and overdominant were determined by PCR.

2.3 Statistical Analysis

The sample size of the study group was calculated using a program at (www.openepi.com/SampleSize/SSCC.htm), adjusted to achieve 80% power and 5% confidence of significance (type I error). Hardy-Weinberg equilibrium for genotype distribution analyses, descriptive statistics, independent t-test, chi-square, and alternative nonparametric tests of the statistical tests, if applicable, were used to analyze the results. $P < 0.05$ was considered significant.

3. RESULTS

One hundred subjects with a positive family history and hundred subjects with a negative family history were enrolled in the study. No gender differences were found between the groups ($P = 0.841$). Also, no significant age differences were detected between the groups as shown in Table 1 and Fig. 1.

Significant differences in genotype frequencies were observed between the +ve family history and -ve family history groups for the codominant, dominant, recessive, and overdominant inheritance models.

In the codominant model, the AA genotype was the most common in the +ve family history group at 51%, while the CA genotype was the most common in the -ve family history group at 42% ($P < 0.0001$), which may be related to the severity of glaucoma in the AA genotype ,but we need more studies. In the dominant, recessive, and overdominant inheritance models, in the +ve family history group, the AA genotypes were all significantly greater than in the -ve family history group ($P < 0.0001$). At last, the mutant A allele was the most common frequent allele in the +ve family history group at 70%, and the least common in the -ve family history group at 39%. This difference was high statistically significant ($P < 0.0001$), as shown in Table 2.

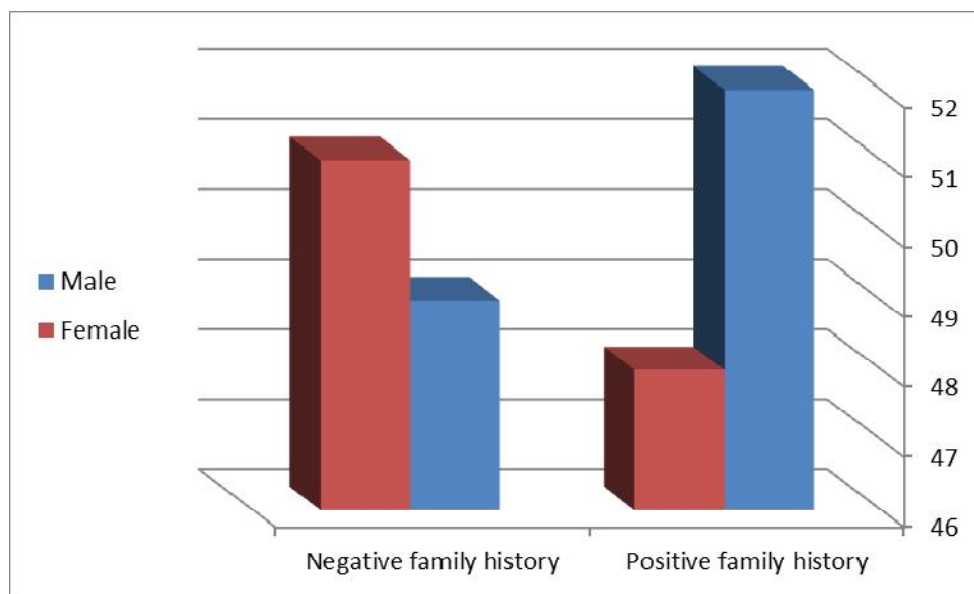


Fig. 1. Sex in both groups

Table 1. Demographic characteristics of both groups

	Sex (%)	Age (Mean+SD)
+ve family history	Male 52 (52%) Female 48 (48%)	45.13±8.13
-ve family history	Male 49 (49%) Female 51 (51%)	45.72±10.97
<i>P</i> value	0.84*	0.18**

*: Chi square test, **: Independent T test

Table 2. Genotype and allele frequencies in both groups

Characteristics		-ve family history	+ve family history	<i>P</i> value
Codominant	CC	40	11	P<0.001*
	CA	42	38	
	AA	18	51	
Dominant	CC	40	11	P<0.001*
	AA-CA	60	89	
Recessive	CA=CC	82	49	P<0.001*
	AA	81	51	
Overdominant	AA-CC	58	62	P<0.001*
	CA	42	38	
Alleles	C	122	60	P<0.001*
	A	78	140	

*: Chi square test

4. CONCLUSION

Many clinical studies have identified family history as a risk factor for POAG [12]. A positive family history of glaucoma in cases of POAG is thought to reflect the influence of genetic variants predisposing to POAG [13]. Some identified the relationship between genetic polymorphism and glaucoma but were insufficient [14]. In this study, the potential relationship between the *TGFB2* rs991967 genetic polymorphism either codominant, dominant, recessive or overdominant and family history of POAG was studied. We found that the *TGFB2* rs991967 polymorphisms were significantly linked with a positive family history of POAG. After a meta-analysis of 12 studies associated with 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 [14]. The present study results show that individuals with the *TGFB2* rs991967 gene polymorphism have a significant association with having a positive family history of POAG. Based on these results, it is likely that the *TGFB2* polymorphisms contribute to the development of the disease. However, the relationship between polymorphism and the severity of the disease need to be further evaluated. Previous studies have explained how *TGFB2* polymorphisms could have a role in the pathogenesis of POAG. Increased levels of activated TGF-β2 in the aqueous humor of

POAG patients [15] are implicated in the fibrosis of the TM [16]. We recommend that other polymorphisms of genes involved in the pathogenesis as well as the quantitative analysis of this gene expression and their relationship with disease risk factors should be further evaluated.

CONSENT AND ETHICAL APPROVAL

The study was approved by the Sohag Faculty of Medicine Ethics Committee, and informed written consent was obtained from all participants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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