·Basic Research·

Association of apolipoprotein E-219T>G promoter polymorphism with primary open angle glaucoma in Turkish population

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Abstract

• **AIM: To investigate the association between apolipoprotein E (*APOE*) -219 T>G promoter polymorphism and primary open angle glaucoma(POAG).**

• **METHODS: Patients and healthy subjects were genotyped with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Genotype/allele frequencies were compared between 122 healthy subjects and in 75 POAG patients using Chi-square test.**

• **RESULTS: Although the frequency of *APOE*-219 GG genotype was higher in POAG group (13.3%) than in control group (6.6%), this finding was not statistically significant (P=0.09). In glaucoma patients carrying GG genotype, mean linear C/D ratio was higher and progression was more compared to glaucoma patients with GT genotype.**

• **CONCLUSION: *APOE*-219 T>G polymorphism does not seem to be a risk factor for the presence of glaucoma, but might play a role in deterioration of the disease, which needs further evaluation.**

• **KEYWORDS:** apolipoprotein E; primary open angle glaucoma; promoter; single nucleotide polymorphism
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INTRODUCTION

Glaucoma is a multifactorial optic neuropathy that affects 70 million people worldwide and is the second leading cause of blindness [1]. It is usually defined by progressive degeneration of retinal ganglion cells and their axons, cupping of the optic nerve head (ONH) and corresponding visual field defects[2].

Primary open angle glaucoma (POAG, OMIM 137760) is the most common form of glaucoma and has a strong genetic component. About 60% of POAG patients have a family history and 10-fold increased risk of POAG was demonstrated for first-degree relatives of affected individuals [3,4]. Several genes, such as *GLC1A* (*myocilin, MYOC, trabecular meshwork induced-glucocorticoid response protein gene, TIGR*), *GLC1E* (*optineurin, OPTN*) and *GLC1G* (*WD repeat domain 36, WDR36*), that increase the risk of POAG have been identified, however the mutations in these genes have been found in less than 10% of the cases[5-8].

Apolipoprotein E (APOE) is the major lipid transporting protein in the central nervous system and takes part in uptaking and redistribution of cholesterol within neuronal network. *APOE*, which have three common isoforms E2, E3 and E4, is encoded by different alleles (ε2, ε3, and ε4). Both *APOE*-219 and *APOE*-491 single-nucleotide polymorphisms (SNPs) were shown to alter transcriptional activity of the *APOE* gene (APOE; OMIM 107741), using promoter activity and electrophoretic mobility-shift assays at molecular level [9]. Allelic forms of *APOE*, and promoter SNPs were associated with elevated plasma levels of cholesterol, increased risk of myocardial infarction and predisposition to Alzheimer's disease (AD) with a possible role in neurodegeneration that occurs AD [10-14]. Some studies have demonstrated increased prevalence of POAG in patients with AD and it has also been reported that patients with AD exhibit optic nerve degeneration and loss of retinal ganglion cells[15-19].

Because both of glaucoma and AD are neurodegenerative disease and have similarities, many studies investigated the association of *APOE* gene with glaucoma. In eye, *APOE* is synthesized in the retina by Müller glial cells, transferred into the vitreous and transported into the optic nerve by retinal

ganglion cells [20]. Recent genetic association studies have implicated the *APOE* gene in the pathophysiology of POAG, but there have been conflicting findings. In the study of Copin *et al* [21], *APOE*-219 G was found to be associated with increased optic nerve damage, as reflected by increased cupping and visual field alteration, whereas, *APOE*- 491T, interacting at a highly significant level with an SNP in the *MYOC* promoter, *MYOC* -1000G, was associated with increased intraocular pressure (IOP) and with limited effectiveness of IOP-lowering treatments in patients with POAG. However, other studies found no association between the *APOE* promoter region polymorphisms and POAG[22-25].

In some studies, the ε4 allele of *APOE* gene was found as a risk factor and ε2 allele as a protective factor for AD and glaucoma [26,27]. In contrast, another studies suggested that *APOE* ε4 allele have a protective effect against normal tension glaucoma (NTG) [23,28]. In our previous study, no significant differences were found in the distribution of *APOE* genotypes between the healthy subjects and POAG patients[29].

The aim of this study was to evaluate the association of the promoter polymorphism -219T>G and POAG and to investigate the possible involvement with the disease phenotype and severity in Turkish patients.

SUBJECTS AND METHODS

Subjects Seventy-five POAG patients (49 women, 26 men) and 122 healthy subjects (67 women, 55 men) were included in this study. All of the subjects have undergone a detailed ophthalmic examination; visual acuity, slit-lamp biomicroscopy, gonioscopy, fundus examination using 90D lens, Goldmann applanation tonometry, ultrasonic pachymetry (POCKET, Quantel Medical), ONH topography (Heidelberg Retina Tomograph HRT II, Heidelberg Engineering, GmBH, Heidelberg, Germany) and visual field analysis. Patients were classified as having POAG, when they had ONH or retinal nerve fiber layer (RNFL) structural abnormalities such as diffuse thinning, focal narrowing or notching of the optic disc rim, especially at the inferior or superior poles, documented progression of cupping of the optic disc, diffuse or localized abnormalities of the peripapillary RNFL, disc rim or peripapillary RNFL hemorrhages, neural rim asymmetry of the 2 eyes consistent with loss of neural tissue and/or visual field damage consistent with RNFL damage (nasal step, arcuate field defect, temporal wedge, or paracentral/midperiphery depression in clusters of neighboring test points), abnormal glaucoma hemifield test (GHT), not explained by any other disease and open angle at gonioscopy, with no secondary causes (such as pigment dispersion, pseudoexfoliation, trauma, uveitis, or steroid induced glaucoma). Linear cup/disc (C/D) ratio was determined according to clinical examination and HRT measurements. Static automated white on white perimetry (SAP) was performed using the 30-2/24-2 full threshold or SITA strategies (Humphrey Field Analyzer HFA II, Carl Zeiss Meditec Inc, Dublin, CA, USA). The visual field was defined as reliable only when the fixation loss, false-positive and false-negative rates were less than 25% . Abnormal visual field was defined as any of the following: GHT result as outside normal limits, pattern standard deviation (PSD) <5%, and the presence of a cluster of 3 contiguous non-edge points in a location typical for glaucoma with P <5%, with at least 1 point having P <1% on the pattern deviation (PD) plot. Glaucoma progression was defined either according to ONH, RNFL and/or visual field changes. Besides clinical examination, topographic change analysis and trend analysis of HRT were used to detect structural changes in ONH over time. Visual field progression is defined as either deepening of an existing scotoma (reproducible depression of a point in an existing scotoma by ≥7 dB), enlargement of an existing scotoma (reproducible depression of a point adjacent to an existing scotoma by ≥9 dB) or development of a new scotoma suggested by a reproducible depression of adjacent 2 or more points by ≥5 dB which are clinically repeatable and consistent in consecutive visual field tests.

Our control group consisted of patients who attended the ophthalmology clinic for refractive errors, routine ophthalmic examination, or medical staff with no ocular problems and no family history of glaucoma. The IOP measurements of the control group were <21 mm Hg. All glaucoma patients and controls were unrelated. The study protocol was in adherence to the tenets of the Declaration of Helsinki and approved by the Ethics Committee of Hacettepe University School of Medicine. Informed consent was obtained from all study subjects after explanation of the nature and possible consequences of the study.

Molecular Genetic Analysis Seventy-five unrelated POAG patients and 122 healthy subjects were genotyped for *APOE* -219T>G promoter region by PCR-RFLP. Genomic DNA was isolated from 400 μL peripheral blood using the phenol-chloroform extraction method.

APOE -219T>G promoter region was amplified using primers 5'-TCC AGA TTA CAT TGA TCC AG-3' (forward) and 5'-AGG ACA CCT CGC CCA GTG AT -3' (reverse). Product was digested with *MboI* restriction enzyme. Samples were checked by 8% polyacrylamide gel electrophoresis. TT was diagnosed as non-carrier of the *APOE* -219T>G promoter polymorphism, GT was heterozygous and GG was homozygous.

Statistical Analysis Statistical analysis was performed by using the SPSS 11.5 Statistical Package Program for

Table 1 Allele and genotype frequencies of *APOE* -219T>G polymorphism in POAG patients and healthy subjects *n* (%)

Genotypes	Healthy subjects			POAG subjects		
	Total (*n*=122)	F (*n*=67)	M (*n*=55)	Total (*n*=75)	F (*n*=49)	M (*n*=26)
GT	114 (93.4)	62 (92.5)	52 (94.5)	62 (82.7)	43 (87.8)	19 (73.1)
GG	8 (6.6)	5 (7.5)	3 (5.5)	10 (13.3)	3 (6.1)	7 (26.9)
TT	0	0	0	3 (4)	3 (6.1)	0
Alleles						
G	130 (53.3)	72 (53.7)	58 (52.7)	82 (54.7)	49 (50)	33 (63.5)
T	114 (46.7)	62 (46.3)	52 (47.3)	68 (45.3)	49 (50)	19 (36.5)

Windows. Genotypic distributions were examined for significant deviation from the Hardy-Weinberg equilibrium by a goodness of fit χ^2 test. The frequencies of genotypes and alleles were compared among glaucoma and healthy subjects using χ^2 test, whereas non-parametric Kruskal-Wallis and Mann-Whitney U tests were used to evaluate the differences in maximum IOP, linear C/D ratio, mean deviation (MD) and PSD values among *APOE* -219 genotypes in POAG group and P<0.05 was considered as statistically significant.

RESULTS

There were 67 women and 55 men in the control group and 49 female and 26 men in POAG. The mean ages of the subjects in POAG and control groups were 63.77±9.5y and 61.75 ±10.1y, respectively (P=0.16). There were no statistically significant differences for age and gender between the two groups(P=0.16 and P=0.18, respectively).

The observed genotypes showed deviation from the Hardy-Weinberg equilibrium in both of the cases or the controls (P>0.05). The distribution of genotypes is given in detail in Table 1. Although the frequency of *APOE* -219 GG genotype was higher in POAG group (13.3%) than in control group (6.6%), this finding was not statistically significant (P=0.09). TT genotype was found in only 3 POAG subjects and none of the healthy subjects. There were no differences in allele frequencies for *APOE*-219T>G between POAG and healthy subjects (P=0.83) (Table 1).

In POAG group, GG genotype was more common in men (26.9%) compared to women (6.1%) (P=0.03); whereas in control group, the frequencies were similar in men (7.5%) and women (5.5%) (P=0.73). Maximum IOP, MD and PSD values were similar in POAG subjects with GT and GG genotypes (P>0.05); however mean linear C/D ratio of POAG subjects carrying GG genotype (0.71±0.18) was higher than patients with GT genotypes (0.56±0.20) (P=0.03). GG genotype was found 21.1% of subjects with progression and in 10.7% of patients without progression (P=0.47).

DISCUSSION

POAG is a progressive neurodegenerative disease with a complex pathogenesis, where multiple susceptibility genes and environmental factors might independently or interactively play roles in the development and course of the disease [28]. In recent years together with the genome-wide association studies, a significant progress has occurred in understanding the genetic basis of POAG [4,7,30,31]. Considering the multigenic characteristics of POAG, polymorphism studies are important for understanding the molecular pathology of diseases and might help to detect a genetic predisposition to POAG. Some SNPs may have a small effect on the disease but interaction with other genes may play important role in the pathogenesis. Also, genetic polymorphisms may help to understand the phenotypic differences between patients and understanding the response to medical treatment[1,8,30,32].

APOE, a major lipid transporting protein in the central nervous system, is thought to play a role in neuronal degeneration. There are several studies investigating the association of *APOE* alleles and promoter polymorphisms with glaucoma in literature. In the study of Copin *et al*[21], *APOE*-219T>G SNP in the promoter region was found to be associated with increased optic nerve damage, whereas, *APOE*-491T, interacting with an SNP in the *MYOC*-1000G, was associated with increased IOP and with limited effectiveness of IOP-lowering treatments in patients with POAG; both having an effect on the phenotype of the disease. Their observations supported the idea of transcriptional regulation of *APOE* expression plays an important role in pathogenesis, independently of allelic forms of *APOE* protein [33]. However, other studies could not show an association between *APOE* promoter region and POAG, consistent with our findings [22-25]. Ressiniotis *et al* [25] were phenotyped 140 POAG cases and 73 controls, and a logistic regression model was used to simultaneously analyze the effect of *APOE* genotype and functional polymorphisms in the *APOE* gene promoter while controlling for potentially confounding variables. They found no evidence of an association between the *APOE* promoter region polymorphisms and POAG. In the study of Fan *et al*[22], *APOE*, -491A>T, -427T>C, -219T>G, and ε2/ε3/ε4 genotype were investigated in a cohort of 400 unrelated

POAG patients and 281 unrelated control subjects. The distributions of -219T>G, -427T>C, and -491A>T were not statistically different between patients with HTG or NTG and control subjects (P>0.05). They identified two interactions between *MYOC* and *APOE*; -83G>A with ε2/ε3/ε4 and IVS2+35A>G with -219T>G and possible gene interactions between *MYOC*, *OPTN* and *APOE*. In another study, the genotypes of the *APOE* polymorphisms in exon 4 and in the promoter at positions -491, -427, and -219 were determined by PCR-RFLP in 294 subjects with high tension glaucoma (HTG), 106 with NTG and 300 unrelated Chinese control subjects [23]. They found no significant difference in the frequencies of *APOE* promoter polymorphisms between POAG patients and control subjects (P >0.0125). When compared with control subjects, the frequency of ε4 carriers was significantly lower in patients with NTG (P =0.008; odds ratio=0.36, 95% confidence interval=0.17, 0.79) but not in HTG (P =0.07). Additionally, Jia *et al* [24] found no association between individual polymorphisms in *OPTN*, *WDR36*, or *APOE* and POAG. We evaluated the association of *APOE*-219T>G promoter polymorphism with POAG in present study. However, no difference was found in genotype/allele distribution between glaucoma subjects and healthy population. In glaucoma group, GG genotype was remarkably higher in men and patients carrying GG genotype had a larger ONH cupping and progressed more compared to the glaucoma patients with GT genotype, which might point a role of *APOE* -219 polymorphism in the progression of glaucomatous damage.

Among the studies, there is not a consensus whether *APOE* (4 allele is a risk factor or protective in the development of POAG [22,23,25]. In our previous studies, we investigated the association of *APOE* gene (ε2, ε3, and ε4 alleles), *p53* codon 72, *p21* codon 31 gene and *myocilin* mt1 (*MYOC* mt1 variant) promoter polymorphisms with high-tension POAG and found no associations between these polymorphisms and development of POAG [29,34]. POAG subjects with *APOE* genotypes ε2/3 and ε3/3 had worse visual field results than subjects carrying ε3/4 genotype[29].

In conclusion, *APOE*-219T>G polymorphism does not seem to be a risk factor for the presence of glaucoma, but might play a role in deterioration of the disease. As the number of subjects in our study is small, further genetic studies on a larger sample of subjects are needed.

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