Association of ARMS2 gene rs10490924 and HTRA1 gene rs11200638 Polymorphisms with Exudative Age-Related Macular Degeneration in Western Aegean Population of Turkey

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ABSTRACT

Purpose: The purpose of the study was to investigate the relation between ARMS2 rs10490924 and HTRA1 rs11200638 polymorphisms, gender and exudative Age-Related Macular Degeneration (AMD) in Western Aegean population of Turkey.

Materials and Methods: *Diagnosis of exudative AMD was* established on the basis of fundus examination, spectral domain optical coherence tomography and fluorescein angiography. Genomic DNA samples were extracted from 75 patients with exudative AMD and 75 healthy controls. Polymerase chain reaction-restriction fragment length polymorphism method was used to determine genotype frequencies of the rs10490924 and rs11200638 polymorphisms.

Results: In the evaluations regardless of gender, thymine risk allele of the rs10490924 was slightly frequent in the exudative AMD group than in controls (p:0.028; OR: 1.291). Women with risk allel for rs10490924 had a higher risk for exudative AMD (p= 0.009, OR= 2.527) than men (p=0.504, OR= 1.233). The disease risk in those with TT genotype was higher in women (p:0.025, OR:5.14) than in men (p= 0.621, OR= 1.54). The risk allele (A) of rs11200638 was significantly associated with the disease in women (p= 0.004, OR= 2.917). Additionally, the frequency of AA genotype was higher *in women patients* (p= 0.016, OR= 4.53) than in men *(p= 0.666, OR= 0.88)*.

Conclusions: To our knowledge, our study is the first one showing that the relation between the rs11200638 and exudative AMD development is more in female gender. We suggest that the relation between rs11200638 polymorphism and this disease be investigated in larger populations.

Key Words: Exudative Age-Related Macular Degeneration, rs10490924, rs11200638, Gender, Polymorphism.

INTRODUCTION

Age Related Macular Degeneration (AMD) is a progressive neurodegenerative disease in the elderly population.[1] AMD is the third cause of blindness in globally and the first cause in developed countries.[2] This disease is staged as early, intermediate, or late.[3] In the early stages, AMD is clinically characterized by the appearance of small drusen (lipid) deposits.[4] There is usually no vision loss. Intermediate-stage AMD consists of extensive medium-size drusens or at least one large drusen, with or without degeneration of the Retinal Pigment Epithelium (RPE). Late stage AMD is subdivided into two clinically distinct types as atrophic (also called non-neovascular or dry form) and exudative (also called neovascular or wet form). Non-exudative AMD consists of geographic atrophy extending into the central macula and drusen. The exudative AMD is caused by the growth of abnormal blood vessels behind the macula and/or a disciform scar.[5]

AMD is a multifactorial disease and implicated risk factors include genetics, age, smoking, ethnicity, hypertension and obesity.[6] There have been a lot of studies about the genetic etiology of AMD. More than 150 AMD-associated variants

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have been reported.[3] CFH located on chromosome 1 and ARMS2/HTRA1 genes located on chromosome 10 are the most associated gene with AMD.[7,8]

ARMS2 rs10490924 polymorphism was examined in Turkish AMD patients, and it was shown that it is a risk factor for the disease.[9,10] However to our knowledge, a potential association between HTRA1 rs11200638 polymorphism and risk of developing exudative AMD has not been studied in the Turkish population. In the current study, we aimed to examine the relation between ARMS2 rs10490924 and HTRA1 rs11200638 polymorphisms, gender, and exudative AMD.

MATERIALS AND METHODS

Patients

75 Patients with exudative AMD and 75 healthy controls were included in this study. People who were included in the study were from the Aegean Region of Turkey, and were not related to each other. This study was approved by the Local Ethics Committee of the Ege University, School of Medicine; and conformed to all norms of the Declaration of Helsinki. Written informed consent was obtained from all participants.

To diagnose exudative AMD, all participants underwent complete ophthalmic examination, including best corrected visual acuity (BCVA) measurements, biomicroscopy, and fundus examination by an ophthalmologist. Fluorescein angiography (FA) and spectral domain optical coherence tomography (OCT) were performed in the patients with exudative AMD (Figure 1). Cases of suspected neovascularization from polypoidal choroidal vasculopathy or retinal angiomatous proliferation were excluded from the study.

Exudative AMD was defined by the presence of at least one of the following features: RPE detachment in one eye; subretinal or sub-RPE neovascular membranes; epiretinal, subretinal, intraretinal, or sub-pigment epithelial scar or glial tissue; intraretinal or subretinal or sub-RPE hemorrhage; hard lipid exudates inside the macular area related to previous findings. The control group comprised patients older than 50 years of age who presented no evidence of AMD. Additionally, induviduals who had any finding of retinal trauma, chorioretinal inflammatory, infective or vascular disease were excluded from this study.

Genotyping

Peripheral blood samples were collected from the patients and control group. Genomic DNA was extracted from leukocytes using the QIAamp DNA mini kit (Qiagen, 51304, Dusseldorf, Germany), according to manufacturer's instructions.

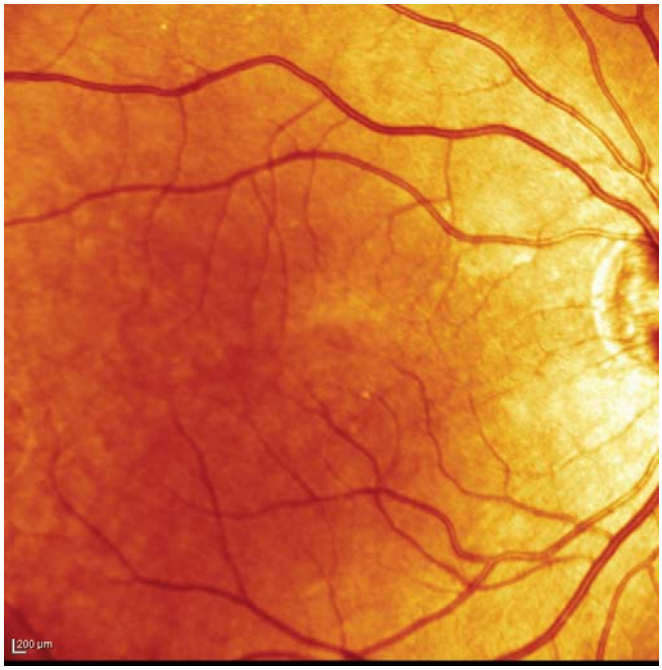

Figure 1: *Color Fundus image of one of the patients with exudative AMD.*

The A69S variant of the ARMS2 gene locus was identified by the primer pair 5' TACCCAGGACCGATGGTAAC3' (forward) and 5' GAGGAAGGGCTGAATTGCCTA 3' (reverse) and the PCR cycling conditions were as described by Pulido et al.[11] The 449 bp fragment generated by PCR was digested with the restriction endonuclease FnuHI (New England Biolabs). It was incubated for 2 hours at 37°C and analyzed through electrophoresis in 2% agarose gel containing 0.5% ethidium bromide by electrophoresis and visualized by UVP BioDoc-It gel image system (UVP Three genotypes, namely GG, GT, and TT, were analyzed by PCR-RFLP. The G (wild type) allele gave bands of 259 and 190 bp. The T (risk) allele gave bands of 449 bp. The PCR products and restriction fragments were separated in 2% agarose gel (Figure 1).

The evaluation of rs11200638 was conducted through PCR and enzymatic digestion, with primer pairs including 5'-ATGCCACCCACAACAACTTT3' and 5'-CGCGTCCTTCAAACTAATGG-3'. The 385 bp fragment generated by PCR was digested by 5 U of the enzyme EagI (New England BioLabs, Ipswich. MA, USA) at 37C for 3 h. The restriction fragments were analyzed through electrophoresis in 2% agarose gel. The G (wild type) allele gave bands of 246 and 139 bp. The A (risk) allele was not digested by the enzyme and gave bands of

385 bp (Figure 1). PCR products of the rs11200638 and rs10490924 were sequenced using an ABI 3130 sequencer (Applied Biosystems, USA) in 15 randomly selected patients to confirm results.

Statistical analysis

Statistical analysis was performed using the SPSS software version 24.0 (SPSS Inc., Chicago, IL, USA). The strength of association between the two polymorphisms (rs10490924 and rs11200638) and exudative AMD risk was assessed by logistic regression analysis. The software Epi Info was used to analyse the data. Odds ratios with corresponding 95% confidence intervals were calculated, with P values <0.05 being considered statistically significant.

RESULTS

A total of 150 cases (75 patients with exudative AMD; 75 controls) were evaluated. There was no significant differences in terms of gender, between the two groups (p= 0.100). The patient group consisted of 28 women (37.3%) and 47 men (62.7%) with a mean age of 68.11 ± 9.563 (mean ±standard deviation). The control group included of 39 women (52%) and 36 men (48%) (mean age 62.23 ±6.086).

The genotype frequencies of ARMS2 A69S (rs10490924) and HTRA1 -512G>A (rs11200638) were in Hardy-Weinberg Equilibrium in the exudative AMD group (ARMS2 A69S; p= 0.155 and HTRA1 -512G>A; p= 0.054) and also in the control group (ARMS2 A69S p= 0.87 and HTRA1 -512G>A; p= 0.204).

The allele and genotype frequency distributions of the rs10490924 and rs11200638 polymorphisms in the patients and control subjects are shown in Table 1 and 2 respectivelly. According to evaluation of ARMS2 A69S (rs10490924), the frequencies of G and T alleles were 44.7% and 55.3%, respectively in the exudative AMD group, whereas in the control, their frequencies were 57.3% and 42.7%, respectively. The A69S thymine risk allele was more frequent in the exudative AMD group than in controls (p= 0.028) and the OR was 1.291 (Table 1). There were not differences between ARMS2 A69S (rs10490924) genotypes in the exudative AMD group (GG 24%, GT

Table 1: *Allele and genotype distributions of the polymorphisms rs10490924 and rs11200638.*

ALL PERSONS IN THE STUDY						WOMEN					MEN				
dbSNP ID	**Allele**	**AMD patients**	**Controls**	**OR (95%CI)**	**P**	**Allele**	**AMD patients**	**Controls**	**OR (95%CI)**	**P**	**Allele**	**AMD patients**	**Controls**	**OR (95%CI)**	**P**
ARMS2 rs10490924	G	67 (44.7%)	86 (57.3%)	1.291 (1.025-1.627)	**0.028**	G	21 (37.5%)	47 (60.3%)	2,527 (1,247-5,119)	**0.009**	G	46 (48.9%)	39 (54.2%)	1,233 (0,667-2,281)	0.504
	T	83 (55.3%)	64 (42.7%)			T	35 (62.5%)	31 (39.7%)			T	48 (51.1%)	33 (45.8%)		
HTRA1 rs11200638	G	60 (40%)	74 (49.3%)	1.176 (0.935-1.480)	0.105	G	16 (28.6%)	42 (53.8%)	2,917 (1,404-6,058)	**0.004**	G	44 (46.8%)	32 (44.4%)	0.909 (0.491-1.684)	0.762
	A	90 (60%)	76 (50.7%)			A	40 (71.4%)	36 (46.2%)			A	50 (53.2%)	40 (55.6%)		

Table 2: *Frequency of genotypes of the polymorphisms rs10490924 and rs11200638.*

ALL PERSONS IN THE STUDY						WOMEN					MEN				
dbSNP ID	**Geno-type**	**AMD patients (n:75)**	**Controls (n:75)**	**OR (95%CI)**	**P**	**Geno-type**	**AMD patients (n:28)**	**Controls (n:39)**	**OR (95%CI)**	**P**	**Geno-type**	**AMD patients (n:47)**	**Controls (n:36)**	**OR (95%CI)**	**P**
c.270G>T (p.A69S) rs10490924	GG	18 (24%)	25 (33.3%)	1.00	0.078	GG	5 (17.9%)	15 (38.5%)	1.00	**0,025**	GG	13 (27.7%)	10 (27.8%)	1.00	0.621
	GT	31 (41.3%)	36 (48%)	1.196		GT	11 (39.3%)	17 (43.6%)	1.94		GT	20 (42.5%)	19 (52.8%)	0.81	
	TT	26 (34.7%)	14 (18.7%)	2.579		TT	12 (42.8%)	7 (17.9%)	5.14		TT	14 (29.8%)	7 (19.4%)	1.54	
HTRA1 rs11200638	GG	16 (21.4%)	21 (28%)	1.000	0.291	GG	5 (17.9%)	12 (30.8%)	1.00	**0,016**	GG	11 (23.4%)	9 (25%)	1.00	0.666
	GA	28 (37.3%)	32 (42.7%)	1.148		GA	6 (21.4%)	18 (46.1%)	0.80		GA	22 (46.8%)	14 (38.9%)	1.29	
	AA	31 (41.3%)	22 (29.3%)	1.849		AA	17 (60.7%)	9 (23.1%)	4.53		AA	14 (29.8%)	13 (36.1%)	0.88	

41.3%, TT 34.7%) and the control group (GG 33.3%; GT 48%, TT 18.7%, p = 0.078) (Table 2).

The frequencies of A allele of the rs11200638 were 60% for exudative AMD, 50.7% for control (p= 0.105) (Table 1). The distribution of the genotypes in the exudative AMD group was 21.4% GG, 37.3% GA, and 41.3% AA versus 28% GG, 42.7% GA, and 29.3% AA in the control group, respectively (p= 0.291) (Table 2). The allele and genotype frequencies of the rs11200638 were not associated with the exudative AMD.

In the evaluations according to gender, it was found that there was a significant relation between the two polymorphisms and exudative AMD disease in women. However, both allele and genotype frequencies of the rs10490924 and rs11200638 polymorphisms did not differ in men (Table 1 and 2). In women, the T allele of rs10490924 was significantly higher in the exudative AMD group than in controls (p= 0.009) and OR was 2.527. The TT genotype was 42.8% in the exudative AMD group and 17.9% in the control group (p= 0.025; OR= 5.14). The A allele of the rs11200638 was significantly increased in the exudative AMD group than in controls (p= 0.004) and the OR was 2.917. The frequencies of the genotypes were 17.9% for GG, 21.4% for GA and 60.7% for AA in the exudative AMD group and 30.8% for GG, 46.1% for GA and 23.1% for AA, in the control group p= 0.016; OR= 4.53)

DISCUSSION

Genetic factors play an important role in both etiology and severity of AMD, with estimates varying from 46% to 71%.[6,12] In our study, we revealed the relationship between the exudative AMD and the ARMS2 rs10490924 polymorphism and rs11200638 polymorphism which are located in the promotor region of HTRA1 in the patient and the control group from the Western Aegean Region. We also evaluated the distribution of these polymorphisms in female and male groups.

It was reported that deficiency of the ARMS2 protein was observed in monocytes of AMD patients with TT genotype of the rs10490924.[13] There is a strong relationship between the ARMS2 rs10490924 polymorphism and exudative AMD; and 8.1-fold increased risk of AMD was reported in individuals with the TT genotype when compared with the homozygous wild-type genotype (GG).[14-16] Significantly different results were reported in the 2 studies conducted in Turkey. Soysal et al revealed 147 patients with AMD and 105 persons with non-AMD. And they did not subclassify this disease in the groups. The mean age of the patients and controls were 70.80±8.6 and 64.63±8.5, respectivelly. They reported that the risk allele of rs10490924 polymorphism was associated with a 3.00-fold increased risk for AMD. The risk of AMD were reported as 8.61-fold in the individulas with TT genotype, when compared with GG genotype.[9] Bardak et al. reported the results of ARMS2 gene sequences in 39 advanced exudative AMD patients and 250 healthy individuals. The mean age of the patient group was 75.7±7.8, but the mean age of the control group was not reported. AMD risk was 39-fold higher in homozygous TT carriers than those with GG genotype.[10] In our study, T allele was associated with nearly 1.3-fold increase in risk for AMD when compared to the wild-type G allele (p= 0.028). However, no relationship was found between the genotypes and the disease. One of the reasons why different results were reported in the studies conducted in Turkey may be because Turkey is a non-homogeneous country with many cultures. The patient and control group were selected from the population in Western Aegean region in our study. One of the other two studies was conducted in Marmara Region, and the other in Aegean Region, close to Central Anatolia.[9,10] Also, this frequency difference might have been occurred because the mean ages of our patient and control group were slightly lower in our study than that of other study groups, and the mean age of our control group was lower than that of the patient group.

Exudative AMD is more common in women than men in the Western population.[17] In 2017, Sasaki et al. investigated the relation between AMD disease and systemic and genetic factors together with the difference of this relation between genders. In that study, the risk alleles of ARMS2 rs10490924 and CFH rs800292 were more frequently detected in women than in men and higher risk of early AMD for women than for men in Asians.[17] In our study, the women with risk allele (T) for rs10490924 had a higher risk for exudative AMD (p= 0.009, OR= 2.527) than men (p= 0.504, OR= 1.233). Additionally, the TT Genotype was 5.14 times higher in female patient group compared to the control group (p= 0.025). No significant relations were detected between the rs10490924 genotypes and the disease in males (p= 0.621).

In patients with AMD, increased HTRA1 expression was shown.[18,19] There were several publications reported the rs11200638 polymorphism as the main variant of HTRA1 associated with susceptibility to exudative AMD. [18,20-22] A allele of the rs11200638 confers a nearly 3.0-fold increase in risk for AMD when compared to the wild-type allele (G).[6,23] An 8.5-fold increased risk of AMD was reported in individuals with the homozygous AA genotype when

compared with the GG homozygotes.[1] There has not been a study conducted in Turkey to investigate the relation between this polymorphism and AMD. In our study, we did not detect a statistically significant association between the HTRA1 rs11200638 polymorphism and exudative AMD regardless gender. However, according to the evaluations based on gender, the women with A allele for the rs11200638 had 2.91-fold higher risk for the disease than those with G allele (p= 0.004). Similarly, the frequency of AA genotype was higher in the women patients (p= 0.016, OR: 4.53) than in men (p= 0.666, OR= 0.88).

In conclusion, the HTRA1 rs11200638 polymorphism was evaluated in Turkish population for the first time. In the evaluations made regardless of gender, no significant relations were detected between this polymorphism and exudative AMD. However, it was shown that HTRA1 rs11200638 and ARMS2 rs10490924 polymorphisms pose a risk for exudative AMD disease in women. This study is the first one showing that HTRA1 rs11200638 polymorphism higher risk of exudative AMD for women than for men. We believe that further studies are necessary to support the relation between HTRA1 rs11200638 polymorphism and this disease in women.

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