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Lack of Association between *ARMS2* Polymorphism and Age-Related Macular Degeneration among Jordanians

Asem M. Alkhateeb[1,*], Duaa A. Abuarqoup[1] and Shadi Q. Al-Khatib[2]

[1]*Department of Biotechnology and Genetics,*

[2]*Department of Ophthalmology, Jordan University of Science and Technology, Irbid, Jordan*

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Abstract

Multiple genetic factors have recently been characterized as risk factors for age-related macular degeneration (AMD). *ARMS2* gene polymorphism, rs10490924, was associated with AMD. This case-control analyzed the role of *ARMS2* gene among Jordanians with AMD. Forty two AMD patients and 84 controls were recruited and genotyped for rs10490924 of *ARMS2* gene. Genotypic distribution of the *ARMS2* polymorphism was not different among AMD patients compared to controls (p=0.20). Allelic distribution was also insignificant among AMD patients (allele frequency 0.33) compared to control (allele frequency 0.23). This study was the first that screen AMD *ARMS2* gene polymorphism among Jordanians. Further studies with larger sample size and different set of patients might be needed to confirm our conclusion.

Keywords: AMD, *ARMS2*, Polymorphism

1. Introduction

Age-related macular degeneration (AMD) is a complex disorder that primarily affects the central region of the retina (macula) and is a leading cause of untreatable visual impairment and legal blindness in older individuals (Fine *et al.*, 2000). The major risk factors for AMD include age, smoking, and family history, with age being the strongest risk factor (Hadley *et al*., 2010). Genetic susceptibility, as indicated by a positive family history of AMD, is associated with a high risk of the disease (Francis and Klein, 2011). Progress has been accomplished in susceptibility loci identification for AMD (Leveziel *et al*., 2011); multiple genetic variants with different environmental triggers might be risk factors that may differ in different populations and genetic backgrounds (Francis and Klein, 2011).

Recently, a single nucleotide polymorphism (SNP), rs10490924, in the age related maculopathy susceptibility 2 (ARMS2) gene that is located on chromosome 10q26 was reported to be associated with an increased risk of AMD in Caucasians and Asians (Dewan *et al.,* 2006; Rivera *et al.,* 2005; Tanimoto *et al.,* 2007). rs10490924 gene polymorphism results in a serine - alanine substitution at codon 69, A69S (Klein *et al.,* 2005). ARMS2 is localized in the ellipsoid region of the photoreceptors within retina, where most of the mitochondria are located (Kanda *et al.,* 2007). ARMS2 is expressed in the retina, but only in primates, consistent with the fact that AMD occurs naturally only in primates (Francis *et al.,* 2008). Although the functional properties of the normal ARMS2 protein are not well known, the risk of rs10490924 gene polymorphism has been shown to alter the encoded protein (Hadley *et al.,* 2010).

Up to our knowledge AMD among Arab population was not investigated. The aim of this study is to examine the possible contribution of ARMS2 SNP as a risk factor among Jordanians.

2. Materials and Methods

2.1. Subjects

Forty-Two Jordanian patients with AMD (mean age 71.6) were enrolled in this study (from 2008 until 2011). The mean age of onset of the disease in our patients was 68.5 years. AMD was diagnosed and graded according to the age related eye disease study (AREDS) trial classification at King Abdullah Hospital in North of Jordan. Ten patients were diagnosed as dry – AMD, thirteen were diagnosed as wet – AMD, and nine patients were diagnosed as dry - wet AMD. Eighty-four ethnically matched controls were recruited from the same hospital. The mean age of controls was 51.4 years of age. In controls there were no signs of early stages of AMD, such as soft drusen or alterations of the retinal pigment epithelium in the macula area, as observed ophthalmoscopically. Signed informed consent was obtained from all subjects before enrolled in this study. Thirty two of participants have completed a short

* Corresponding author. e-mail: asemalkhateeb@just.edu.jo.

questionnaire about demographic data, some of which are presented in Table1.

Table 1. Baseline characteristics of age-related macular degeneration patients and controls

Variable (s)	Cases (N=32)	Controls(N=84)
Infection Status		
No AMD	-	84
Dry AMD	10 (31.0%)	-
Wet-AMD	13 (41.0%)	-
Both	9 (28.0%)	-
Sex		
Male	21 (74.0%)	23 (27.4%)
female	11 (26.0%)	61 (72.6%)
Mean age	71.6	51.4

2.2. Genotyping

Peripheral blood samples were collected from all participants. DNA was extracted using DNA purification Kits (Qiagen, Hilden, Germany) according Manufacturer's protocol. An amplicon was amplified by Polymerase Chain Reaction (PCR). Primers were designed according to Rozen and Skaletsky (2000), forward primer: 5'-CTCTGCGAGAGTCTGTGCTG-3' and reverse primer: 5'-GGGGTAAGGCCTGATC- ATCT-3'. Amplifications were performed in 25 µl volume using the thermal cycler manufacturer. Each PCR reaction contained 12.5 µl GoTaq Green Master mix (Promega, Madison, WI, U.S.A.), 1 µl of each primer (1 µM final concentration) (Alpha DNA, Montreal, QC, Canada), 9.5 µl nuclease free water, and 1 µl of DNA template (nuclease free water added up to 25 µl). After the initial step of 5 minutes at 95o C, the samples were processed through 14 cycles from 59o C (decreasing 0.5ºC in each cycle) to 56ºC. At 56ºC, the samples were processed through 25 temperature cycles of 30 seconds at 94ºC, 30 seconds at 56ºC, and 30 seconds at 72ºC, and a final extension step of 72ºC for 10 minutes. PCR product were run on 2 % agarose gel and visualized under UV light after ethidium bormide staining to assess the correct size of the amplified products. Product then digested with 5u of ApeKI enzyme (Fermentas, St. Leon-Rot, Germany), at 37ºC. Restriction digested PCR products were then separated by 2 % Agarose gel electrophoresis. Fragments of 97bp, 71bp, and 54bp were detected for the GG homozygote wild type (presence of ApeKI restriction site). Fragments of 168bp and 54bp were detected for the TT mutant homozygote (absence of ApeKI restriction site). TG heterozygous combined the four fragments of 168bp, 97pb, 71 bp and 54bp (Figure 1). Positive and negative controls were included with each run.

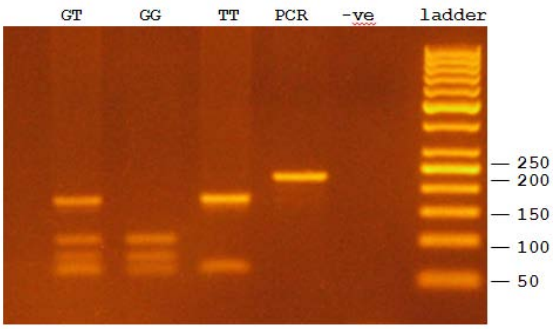

Figure 1. Gel electrophoresis of DNA digested by *ApeKI* showing the different genotypes of the *ARMS2* rs10490924

2.3. Statistical analysis

Data were analyzed using the *OpenEpi* program version 2.3.1, for the calculation of allele and genotype frequencies. The genotype frequencies were assessed by Chi-squared test to determine if they obey Hardy-Weinberg equilibrium (HWE) at the SNP locus.A p value <0.05 was considered as statistically significant. Odds ratios and 95% confidence intervals were also calculated.

3. Results

Forty-two patients with AMD and 84 ethnically matched controls were genotyped. Genotypic frequencies of the ARMS2 SNP in the control population met HWE expectations (no significant difference was found between observed and expected genotype values). The frequency of the heterozygous genotype (GT) was 28.6% (24 of 85) and of the homozygous variant genotype (TT) 8.3% (7 of 85) in the control group and this corresponded to an allelic frequency of 22.6% (table 3). In AMD patients, the variant T allele was detected in 28.6% as a heterozygous GT genotype and 19.0% as a homozygous variant genotype; this corresponded to an allelic frequency of 33.3%. Statistical analysis showed no significant allelic or genotypic (p=0.20) nor allelic (p=0.068) difference between vitiligo patients and controls.

Table 3. Allele frequency of *ARMS2* – rs10490924 in controls and patients

Allele Frequency n (%)				
	Patients	Control	*p*-value	Odds ratio (95% CI)
T	28 (33.3%)	38 (22.6%)	0.068	1.71 (0.96-3.06)
G	56 (66.7%)	130 (77.4%)		

4. Discussion

Many gene variants have been implicated in AMD; the two major ones are complement factor H (*CFH)* SNP (rs1061170) (Klein *et al*., 2005; Haines *et al*., 2005; Edwards *et al*., 2005) and *ARMS2* SNP (rs10490924) (Rivera *et al*., 2005; Jakobsdottir *et al*., 2005). ARMS2 function is still unclear, but whether the mRNA transcript is translated is debated. Furthermore, the predicted ARMS2 protein does not show homology with other proteins.

Ethnicity influences disease risk depending on the genetic background (Hardy *et al*., 2003). *ARMS2* SNP has varying frequency within different populations. East Asia; China, Japan and India have an allele frequency approaching 40% which significantly differs from European in which the risk allele frequency approaches 20% (Table 4). This may reflect different AMD subtypes among East Asian populations.

Table 4. rs10490924 allele frequency among different populations (normal unaffected individuals)

Population	N	Minor allele (T) frequency	Reference
China	93	0.387	Lee *et al*., 2008
Japan	94	0.378	Kondo *et al*., 2007
India	203	0.358	Kaur *et al*. ,2008
Jordan	84	0.226	Present study
France	116	0.22	Leveziel *et al*., 2007
USA	280	0.22	Kanda *et al*.,2007
Sephardic Jews	40	0.212	Chowers *et al*., 2008
UK	266	0.205	Hughes *et al*. ,2007
Arabs in Israel	10	0.2	Chowers *et al*., 2008
Germany	612	0.196	Rivera , *et al*., 2005
Ashkenazi Jews	68	0.192	Chowers *et al*., 2008

ARMS2 region on chromosome 10q26 had multiple variants that are in linkage disequilibrium which makes the identification of the true causal variant uneasy (Hadley *et al*., 2010). Two other variants in linkage disequilibrium with rs10490924 are rs11200638 in the promoter of *HTRA1* (Dewan *et al*., 2006; Yang *et al*., 2006) and an insertion deletion in the 3'-UTR region of *ARMS2* (Fritsche *et al*., 2008). Both of these variants are candidate for causal effect on AMD.

Middle East constitutes a genetically divergent population (González *et al*., 2008). Till now no genetic studies screened Middle East AMD patients and none examined the contribution of *ARMS2 as* a risk factor for AMD. The only study conducted on AMD Arabs was with a subset of only ten Arab patients and ten controls without reliable data (Chowers *et al*., 2008). This study included 42 patients without any significant association between the SNP among AMD patients.

In conclusion, this study shows no association between *ARMS2* SNP and AMD among Jordanians. Lack of association might suggest other genes might play a prominent role like the complement factor gene *CFH*. Screening *CFH* in our patients and/or enrolling a bigger set of AMD patients in this population may shed more light on the AMD among Jordanians

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