Relationship Between Three Common Genetic Variations of *ATM* and the Risk of Age-Related Macular Degeneration

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ABSTRACT

Purpose: The association of a few single nucleotide polymorphisms (SNPs) of gene involved in DNA repair pathways with the risk of age-related macular degeneration (AMD) have been studied. However, the contribution of SNPs of ataxia telangiectasia mutated gene (*ATM*; MIM: 706585) has not yet been evaluated. The aim of this study was to examine the relationship between the rs611646, rs609429, and rs228593 polymorphisms of the *ATM* with the risk of AMD.

Materials and Methods: In the present study we used two pooled genomic DNA samples of 120 AMD patients and 118 healthy controls. PCR was carried out by specific primers using pooled samples, as template DNA. The allelic frequencies were estimated using measurement of band intensity on agarose gel electrophoresis.

Results: Statistical analysis revealed that there was no significant association between the studied *ATM* polymorphisms and the risk of AMD.

Conclusions: This study suggested that the rs611646, rs609429, and rs228593 of *ATM1* is not predisposed polymorphisms for AMD.

Keywords: Age-related macular degeneration, *ATM*, Pooled samples.

INTRODUCTION

Age-related macular degeneration (AMD) is a progressive loss of photoreceptors predominantly in the central retina, which is the leading reason of visual impairment and blindness in the developed countries. AMD is a multifactorial disease, it means that both genetical and environmental factors contribute to its development.[1]

Ataxia telangiectasia mutated gene (*ATM*; MIM: 706585) plays an important role in DNA repair and genome stability. The ATM protein has serine/threonine kinase activity and activates over a hundred proteins involved in numerous cellular functions, such as DNA damage response and other cellular responses like DNA double strand breaks repair.[2] Loss of function of ATM results ataxia-telangiectasia (an autosomal recessive disease).[3] Epidemiological data demonstrate an increased risk for several types of cancers in persons having single copy of the above-mentioned recessive allele.[3, 4] The *ATM* gene, in addition to the identified mutations in relation to ataxia-telangiectasia, has also a large number of single nucleotide polymorphisms (SNPs). It is known that some genetic variations can potentially cause alter gene expression, therefore, they might be functional.[5, 6] Based on some evidences the rs611646, rs609429, and rs228593 are functional SNPs and probably alter the *ATM* gene expression.[7-9] Molecular epidemiological studies have indicated significant associations between *ATM* SNPs and different types of cancer.[7, 10-13]

Oxidative stress may play a key role in the pathogenesis of AMD and inducing damage in biomolecules, including DNA molecule.[14] A few studies have attempted to evaluate the association between polymorphisms in some DNA repair genes and the risk of AMD.[15-18] No study has investigated the association between the *ATM* polymorphisms and susceptibility to AMD. As the *ATM* rs611646, rs609429, and rs228593 SNPs are common in gene pools and potentially they have immediate functional significance, the present study was carried out.

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MATERIALS AND METHODS

In the present hospital-based case-control study, 120 AMD patients (75 males, 45 females) and 118 healthy controls (68 males, 50 females) were included. Detailed description of the participants has been reported in our previous article.[17] The study was approved by the local ethics committee. The study was conducted in accordance to tenets of Helsinki Declaration. Informed consent was obtained from all participants.

Here, we used pooled DNA samples for estimating the allelic frequencies of the polymorphic sites, as described previously.[19] For each polymorphism, a heterozygous individual was considered as calibrator sample. To do this, two pooled DNA samples of AMD patients and healthy controls were prepared and used as template DNA in PCR. The primers used for PCR were showed in Table 1. For each polymorphism, estimation of allelic frequencies was repeated three times and the average value was used. Figure 1 shows the bands of a heterozygote genotype and pooled samples of control subjects and AMD patients for each studied genetic polymorphisms, which used for estimation of allelic frequency by measured intensity of bands.

The associations between alleles of the studied SNPs polymorphisms and the risk of AMD were estimated by the odds ratios (ORs) and 95% of confidence intervals (95% CI) using unconditional logistic regression in SPSS software package version 25. A $P<0.05$ was regarded statistically significant.

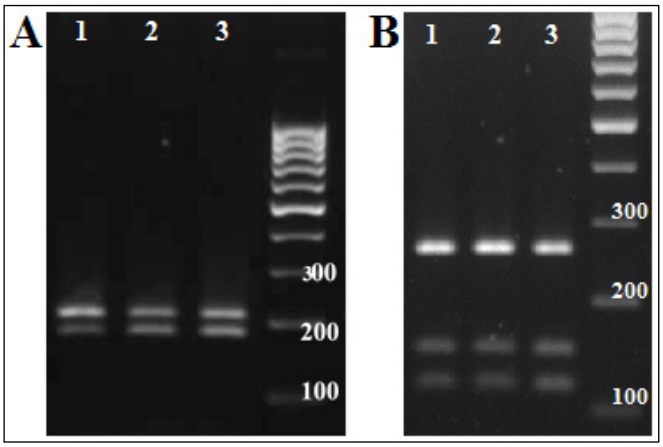

Figure 1: *Gel electrophoresis of PCR products after digested by restriction enzymes of a heterozygote individual (lane 1) and pooled samples of AMD patients (lane 2) and control participants (lane 3) for the rs609429 (A) and rs225893 polymorphism (B). In both panels the last lane is 100bp DNA ladder.*

RESULTS AND DISCUSSION

Table 2 summarized the allelic frequencies of the studied SNPs in AMD patients and healthy controls. Statistical analysis revealed that there was no correlation between

Table 1: *Primers used for genotyping.*

SNPs	Primers (5'→3')	Annealing temperature (°C)	Restriction enzymes	Band size (bp)
rs611646	**F:** GTTACTTTACCTTCCAATGG **R:** ATCCTGTGATCCTACTAGAT	51	*Hinf*I	A: 356 T: 220, 136
rs609429	**F:** CTCAATTTCCTGGTTATAAAATGAGAAGGTAC **R:** TTAACTACTTGTCAGGGACTATCTTAAGGAC	57	*Kpn*I	G: 207 C: 172
rs228593	**F:** TCCCTGCCCATGATTATCTC **R:** GCCTTCGTACCCTCA TCAGT	56	*Bcl*I	A: 264 G: 146, 118

Table 2: *Association between three common ATM polymorphisms and the risk of age-related macular degeneration.*

Polymorphisms/Alleles	Controls (%)	Cases (%)	OR	95% CI	*P*
rs611646					
A	115 (48.77)	102 (42.41)	1.00	–	–
T	121 (51.23)	138 (57.59)	1.28	0.89-1.84	0.173
rs609429					
G	72 (30.33)	70 (29.02)	1.00	–	–
C	164 (69.67)	170 (70.98)	1.06	0.72-1.57	0.749
rs228593					
A	97 (40.98)	118 (49.11)	1.00	–	–
G	139 (59.02)	122 (50.89)	0.72	0.50-1.03	0.077

the *ATM* polymorphisms and the risk of AMD. Based on our knowledge, this is the first study on the relationship between *ATM* polymorphisms and the risk of AMD.

Given that the AMD is significantly associated with oxidative stress [20] and oxidative stress can induced DNA damage,[14] it was expected that AMD is associated with SNPs in DNA repair genes. The association of AMD with some genetic variations in genes involved in DNA repair pathways has already been shown.[15-18] Therefore, our prior hypothesis was that SNPs of *ATM* are associated with susceptibility to AMD. The present findings are in disagreement with the above-mentioned previous studies and did not support our hypothesis. At this time, it is not possible to comment definitively and we will have to wait for further studies from other countries with larger sample sizes.

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