

The Association of SMUG1 Gene Polymorphism with Age-related Macular Degeneration in Northwestern Iran

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Abstract

Age-related macular degeneration (AMD) is the most common cause of irreversible vision loss and debilitating disease in old age, which involves the central retina/macula among elderly patients. The genetic and environmental factors have important role in this multifactorial disease. Oxidative stress and DNA damages would have important impact on the onset and progression of AMD. In this study, the possible association of c.-31A>G (rs3087404) polymorphism in the promoter region of SMUG1 gene with AMD disease was investigated. Fifty five AMD patients and 130 healthy age-, gender- and ethnicity-matched unrelated people as control group were genotyped by restriction fragment length polymorphism PCR (RFLP-PCR). Both groups were from Northwest of Iran (Tabriz). Statistical analysis showed a significant association of AG genotype of this polymorphism with AMD. These results suggest a possible protective effect of this genotype for AMD disease ($P=0.02$, $OR=0.574$) among patients from Northwest of Iran. This genotype was observed more frequently in controls compared to the patients (59.23% v s 45.45%).

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Introduction

Age-related macular degeneration (AMD) is one of the most common irreversible causes of severe loss of vision in the elderly population over 60 years (1). Studies have shown that 8.7% of the worldwide population has age-related macular degeneration, and the projected number of people with the disease will be around 196 million in 2020, increasing to 288 million in 2040(2).

Clinically, AMD is divided into two main forms of the disorder i.e. the dry and wet forms. The dry form

refers to the confluent atrophy of the choriocapillaris and associated retinal pigment epithelium (RPE) and the wet form is linked to choroidal neovascularization, an ingrowth of new vessels occurs from the choriocapillaris invading the retina, which may result in a sudden loss of central vision and emerge as the most rapidly progressing form of AMD(3). AMD is a highly complex disease with environmental and genetic risk factors. There are several factors affecting AMD, such as age, smoking, gender, UV exposure,

female sex, obesity, race, diet, smoking, cardiovascular disease and unbalanced diet(4,5). Over the recent years, genetic studies identified single nucleotide polymorphisms (SNPs) which confer increased or decreased risk of disease and SNPs associated with inflammation, oxidative stress, angiogenesis, and other pathological processes have been linked to AMD. The oxidative stress affects the DNA of RPE cells, which promotes genome instability in these cells (6). These effects contribute to decrease in the efficacy of DNA repair with age. Therefore individuals with DNA repair impaired may be more susceptible to AMD if oxidative stress affects their RPE cells (7).

The retina is highly susceptible to oxidative stress secondary to the elevated oxygen partial pressure, high metabolic activity, prolonged exposure to UV and blue light, the presence of photosensitizers, and oxidative phagocytosis of photoreceptors(8). Oxidative stress was also reported with the appearance of uracil in DNA. The presence of uracil in DNA leads to genomic instability; therefore, precise mechanisms for the removal of uracil from DNA and prevention of its incorporation are essential for maintaining DNA integrity (9). Under normal conditions, such lesions are repaired by the base excision repair pathway, which is initiated by "uracil-DNA glycosylases" (UDGs). Four uracil glycosylases excise uracil from DNA in humans: UNG, SMUG1, MBD4, and TDG. In spite of its name, SMUG1 (single-strand selective monofunctional UDG) also known as FDG; UNG3; HMUDG, removes uracil from double-stranded DNA and single-stranded DNA (10,11). The purpose of this study was to investigate the possible association of SMUG1 (c.-31A.G) gene polymorphism located in promoter region with the risk of AMD.

Materials and Methods

- Subjects

This study included 55 patients with AMD and 130 individuals without AMD (control group). All patients and control people that were of age 50 or older were examined in the Nikookari Eye Hospital of Tabriz. The exclusion criteria included any retinal diseases other than AMD such as high myopia, retinal dystrophies, central serious retinopathy and systemic inflammatory disease. The controls were included with absence of diagnostic criteria for AMD or other retinal abnormality. All participants underwent a standard ophthalmic examination.

DNA extraction and SNP genotyping analysis

Genomic DNA was extracted from peripheral white blood cells of whole-blood samples using standard laboratory protocols(12). All 185 DNA samples were successfully genotyped by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) technique. To identify the allelic variant of PCR products, some samples were chosen at random by direct sequencing (Figure1). The primer sequences, were forward: TGGCTCTGGGGCGACTTT and reverse: AGAGCCACAGGCTGGAAGTCAAT. The amplified SMUG1 fragment containing a polymorphic site was digested with restriction enzyme PstI(13). Agarose gel electrophoresis of the PCR products and digested PCR products are shown in Figure 2.

Statistical analysis

The comparison of alleles and genotype frequency between patients and healthy controls was performed using the Chi-square and Fishers exact test. *P* values less than 0.05 were considered as statistically significant.

Results

We performed analysis of the genotypes of the c.-31A>G polymorphism of the SMUG1 gene, in the control group and AMD patients.

Subjects included 55 AMD patients with a mean age 74.5 ± 7.66 years old and 130 healthy controls with the mean age 72.27 ± 6.43 years. The genotype distributions in AMD controls were GG (21.53%), AG(23.59%), AA(19.23%) and in AMD cases: GG(29.09%); AG(45.45%) and AA(25.45%). The genotype and allele distributions are presented in Table 1. Results of the present study showed a significant association of AG genotype of this polymorphism with AMD.

Fig1: Direct sequencing of the c.-31A>G polymorphism in SMUG1

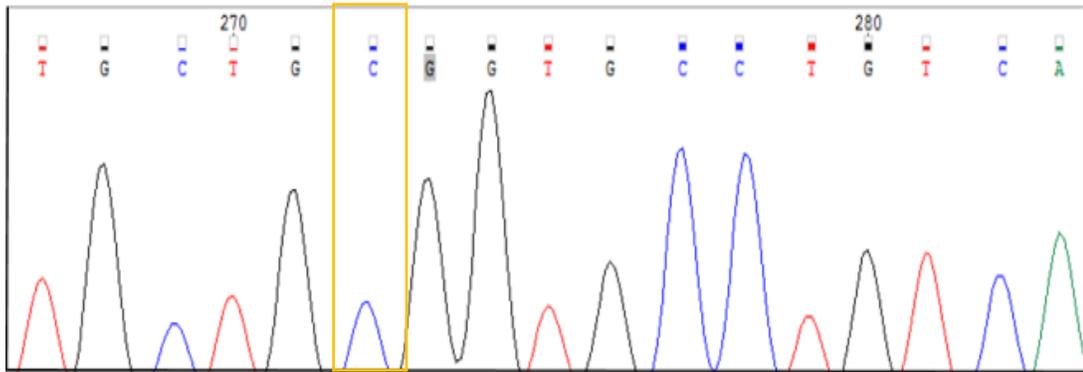


Fig 2: PCR-RFLP data after enzyme digestion by PstI on electrophoresis gel for SMUG1 gene. 1: DNA Ladder; 2: PCR product; 3: GG homozygous; 4: AG heterozygous and 5: AA homozygous.

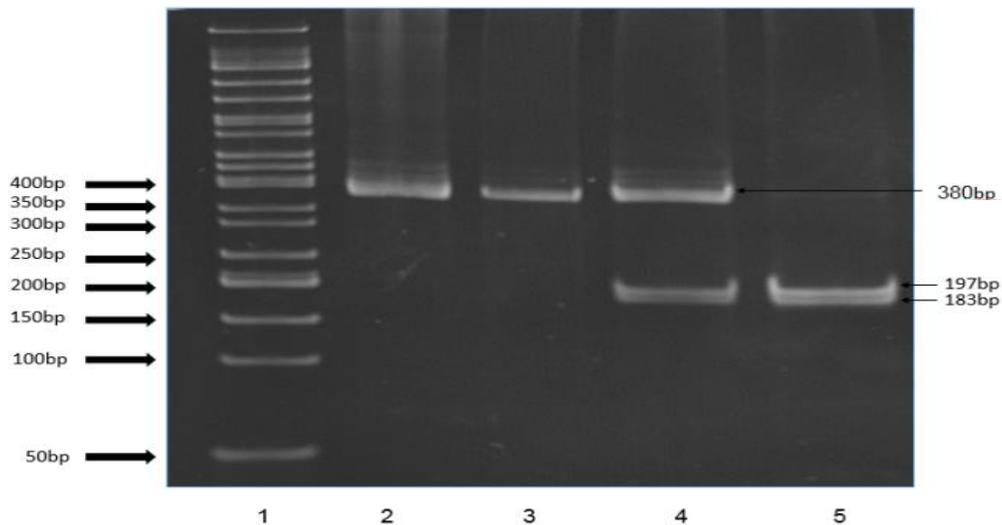


Table 1: The genotype and allele distribution among AMD patients and the control group

Genotype	Control(130)	AMD(55)	OR	P-value
GG	28(21.53%)	16(29.09%)	1.495	0.08
AG	77(59.23%)	25(45.45%)	0.574	0.02
AA	25(19.23%)	14(25.45%)	1.434	0.13
Allele				
A	127(48.84%)	53(48.18%)	0.974	0.49
G	133(51.15%)	57(51.81%)	1.027	0.50

Discussion

The pathogenesis of AMD is complex and involves interactions of environmental and genetic factors (14). It is shown that the oxidative stress plays an important role in different processes inducing biomolecules damaging such as DNA(15).

The impaired efficacy of DNA repair, which is decreased with age, might combine with enhanced sensitivity of retinal pigment epithelial cells to blue and UV lights, contributing to the pathogenesis of AMD(7). It has been suggested that polymorphisms in DNA repair genes reduce their efficiency to repair DNA damage and thereby lead to a high susceptibility to age-related eye diseases(16).

SMUG1 protein is involved in base excision repair pathway, and its proper function may be significant for maintaining cellular homeostasis in retinal cells. The c.-31A>G polymorphism is located in the noncoding, regulatory regions of the gene and can influence the activity of the transcriptional regulatory region, which may have an impact on gene splicing, transcription factor binding, or noncoding RNA. It has been suggested that uracil-processing gene polymorphisms can lead to altered enzyme activity and uracil concentrations, increasing uracil mis-incorporation, and, in this way, contribute to human diseases (17).

One study demonstrated that two SNPs in the SMUG1 (rs2029166 and rs7296239) and UNG (rs34259) genes associated with an increased blood uracil DNA concentration among carriers of the variant genotype, whereas a SNP in the DUT gene (rs4775748) associated with a decreased uracil DNA concentration(18). The results of some studies suggest that uracil for two reasons, the generated in significant amounts in DNA after oxidative stress and the presence of uracil in DNA occurs as a result of deficiency of vitamins, may play a role in the pathogenesis of AMD(19,20). Another study observed that the g.4235T>C (rs2337395) and c.-31A>G (rs3087404) polymorphisms in UNG and SMUG1 genes, could be associated with susceptibility to develop AMD(13).

In this study we investigated the possible association between alleles and genotypes of SMUG1 and AMD. The results showed an association between AG genotype of the SMUG1 gene polymorphism and AMD. Our findings showed that heterozygous individuals who carry the AG genotype represent a lower risk of AMD. Therefore this genotype might retain a protecting role against AMD disease.

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