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Research Article

Screening for a Deleterious *STING1* Polymorphism and its Association with Age-Related Macular Degeneration: A Case-Control Study

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ABSTRACT

Objectives: Inflammation is involved in development of age-related macular degeneration (AMD), with the cGAS-STING pathway playing a critical role. This pathway activates in response to cytosolic DNA, such as accumulated mitochondrial DNA from aging or oxidative stress. Given STING's encoded by the *STING1* gene, we hypothesized that functional *STING1* polymorphisms might influence AMD susceptibility.

Methods: To identify the most relevant polymorphism, all polymorphisms of *STING1* with MAF ≥1% were extracted from NCBI. The rs7380824 was selected for genotyping as it demonstrated the highest PSI value—a novel metric combining CADD score and MAF—indicating high potential deleteriousness. Then a hospital-based case-control study comprised 237 subjects (122 AMD patients, 115 controls) was carried out to investigate the association between the rs7380824 and the risk of AMD. Genotyping employed PCR-RFLP, and statistical analyses used logistic regression adjusted for age, smoking, and workplace exposure.

Results: The genotypic frequency of the rs7380824 polymorphism was in Hardy-Weinberg equilibrium. No significant association was found between the genotypes of this polymorphism and AMD risk. However, a borderline protective effect was observed for the TT genotype versus CC+CT (OR=0.19, 95% CI: 0.03-1.16, p=0.073) after adjusting for age, workplace and smoking habits of the participants.

Conclusion: As the first investigation of the association between the *STING1* polymorphism (rs7380824) and AMD risk, this study did not identify a significant overall association. However, the observed protective effect of the TT genotype, potentially covered by the limited sample size, highlights the necessity for validation in studies with larger samples.

Keywords: Age-related macular degeneration; AMD; Polymorphism; rs7380824; *STING1*

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Introduction

Substantial evidence points to the important role of inflammation in the pathogenesis of age-related macular degeneration (AMD) (1). The stimulator of interferon response CGAMP interactor 1 (STING) protein, encoded by the *STING1* gene (OMIM: 612374), is primarily located in the endoplasmic reticulum membrane and plays a key role in activating the innate immune response, particularly by inducing type I interferon production in response to cytosolic DNA (2).

Multiple lines of evidence indicate that the cGAS–STING pathway is activated in response to damage caused by risk factors in the retina (3-6). With aging as the most significant risk factor, retinal pigment epithelium (RPE) cells, become more susceptible to DNA damage, affecting both nuclear and mitochondrial DNA (mtDNA). Due to its lack of histone protection and limited repair capacity, mtDNA is highly vulnerable to oxidative stress. Furthermore, declining autophagy with age leads to the accumulation of damaged molecules, including mtDNA, within the cell. Consequently, these damaged DNA fragments enter the cytosol and are recognized as danger signals (7, 8). In response, the expression of the *STING1* gene and the chromatin accessibility of the *cGAS* and *STING1* promoters increase leading to activation of the cGAS–STING pathway (5). In addition to aging, intense sunlight also activates this pathway, which is associated with increased STING1 expression in activated macrophages and microglia resulting in exacerbation of the photoreceptor cell death (9).

The etiology of AMD involves a complex interaction of inflammatory, oxidative, degenerative, and genetic components (10). Several studies have investigated the association between genetic variants and the risk of AMD in different populations. For instant, polymorphisms within the genes encoding proteins of the alternative pathway of complement (11), toll-like receptor 4 (12) and vascular endothelial growth factor were associated with the risk of AMD (13). Although the role of STING1 gene in the pathogenesis of AMD has been previously reported, no study has investigated the association between the variant of this gene with the risk of AMD. Therefore, the present study has two objectives. First, to identify a potential risk-associated polymorphisms in the *STING1*, and subsequently, to investigate the association of this polymorphism with the risk of developing AMD.

Materials and Methods

All single nucleotide polymorphisms (SNPs) of the *STING1* with a minor allele frequency (MAF) of at least 1% were extracted from the NCBI SNP database. To identify high-risk polymorphisms, the recently introduced polymorphism selection index (PSI) index was used (10). This index is calculated by multiplying the combined annotation dependent depletion (CADD (score by the MAF. CADD is a metric for assessing the deleteriousness of polymorphisms, estimating their potential risk using information such as evolutionary conservation and functional effects (14). A polymorphism with the highest PSI value was selected to investigate its association with AMD.

This is a hospital-based case-control study. The samples used in this study are the same as those used in the previous study (15), with the difference that two patients were added to the patient group, and genotyping was not possible for three subjects from the control group. Therefore, the present study was conducted on 237 participants, comprising 122 patients (47 women, 75 men) and 115 healthy individuals (48 women, 67 men). Participants were collected from the ophthalmology clinics of Khalili Hospital and Pars Hospital in Shiraz. It should be noted that while there was no significant difference in gender distribution between the two groups ($p = 0.614$), the difference in their mean age was statistically significant (69.5 ± 9.7 years in patients vs. 63.4 ± 10.1 years in controls, $p < 0.001$). This study was approved by the Bioethics Committee of Shiraz University (IR.US.PSYEDU.REC.1403.038). Informed consent was obtained from all participants involved in the study.

DNA was extracted from blood samples using the boiling method. Genotyping for the rs7380824 polymorphism was performed using the restriction fragment length polymorphism (RFLP) method. Primer sequences were designed using Oligo7 software: STING1-F: 5’ ATAGCCCCTTCTGACTCTTTGG 3’ / STING1-R: 5’ CTTGAGCAGGCCAAACTCTTC 3’. The PCR reaction for genotyping was carried out for 35 cycles, with initial denaturation step at 95°C for 5 minutes, then denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds and a final extension at 72°C for 10 minutes. Following the PCR reaction, the resulting fragment length was 184 base pairs. For enzymatic digestion, the *Msp*I enzyme was used. Successful digestion by the enzyme yielded fragments of 158 and 26 base pairs; however, the 26 bp fragment was too small to be visible on the gel.

Statistical analysis

Data analysis was performed using various statistical tests, including Chi-square, independent *t*-test, and logistic regression. Odds ratios (OR) with 95% confidence intervals (CI) were used to examine the association between genotypes and the risk of AMD. A *p*-value of less than 0.05 was considered statistically significant. All data analyses were conducted using SPSS software version 26.

Results

The *STING1* gene had fifteen single nucleotide polymorphisms with a minor allele frequency greater than 1%. Among them, there was one synonymous, four missense, eight intronic, one in the 5' untranslated region, and one upstream polymorphism. The PSI values for these polymorphisms were calculated, as shown in Table 1. The rs7380824 polymorphism had the highest PSI value, indicating that this polymorphism has a higher probability of causing deleterious effects and is a more suitable candidate for genetic association studies with diseases. Consequently, the association of this polymorphism with AMD was investigated.

The results concerning the genotypic frequencies in the study groups are presented in Table 2. A comparison of the observed and expected genotype frequencies (based on the Hardy-Weinberg equilibrium) revealed no significant difference between them ($\chi^2 = 0.73$, df = 1, $p = 0.390$). The results of the statistical analysis, considering the CC genotype as the reference, indicated that the CT (OR = 0.96, 95% CI: 0.55-1.67, $p = 0.894$) and TT (OR = 0.25, 95% CI: 0.05-1.26, $p = 0.094$) genotypes did not show significant association with the risk of AMD (Table 2).

As mentioned in methods section and our previous study (15), the control and patient groups had a statistically significant difference regarding smoking habit and workplace. Therefore, in order to rule out the possible confounding effect of these variables on the association between genotypes and the risk of AMD, we used multivariate logistic regression analysis. Our analysis indicated that although the adjusted OR for the TT genotype compared to other genotypes suggested a reduced risk of AMD, it showed only a marginal level of significance (OR = 0.19, 95% CI: 0.03-1.16, $p = 0.073$).

Discussion

Chronic inflammation is considered as a key factor in the development and progression of AMD (13). Within this context, the STING protein, as a crucial component

Table 1. Polymorphism selection index (PSI) for polymorphisms in the *STING1* gene with MAF > 0.01

SNP	Type	Alleles	MAF	CADD GRCh38-v1.7	PSI
rs7380824	Missense Variant	C>T	0.2750	24.90	6.848
rs11554776	Missense Variant	C>T	0.2031	14.48	2.941
rs75746446	Intron Variant	T>C	0.1753	13.74	2.409
rs1131769	Missense Variant	T>C	0.1272	12.52	1.593
rs13166214	2KB Upstream Variant	A>G	0.4333	1.657	0.718
rs7447927	Synonymous Variant	C>G	0.4285	0.912	0.391
rs73257329	Intron Variant	C>G	0.2370	1.640	0.389
rs7380272	Intron Variant	C>T	0.2650	1.364	0.361
rs80059114	Intron Variant	G>A	0.0433	2.474	0.107
rs73257333	Intron Variant	T>C	0.0208	4.021	0.084
rs78233829	Missense Variant	C>G	0.2680	0.214	0.057
rs79691341	5 Prime UTR Variant	C>T	0.0246	1.933	0.048
rs116583357	Intron Variant	G>A	0.0102	0.549	0.006
rs151074578	Intron Variant	C>G	0.0112	0.246	0.003
rs185552744	Intron Variant	C>T	0.0122	0.087	0.001

Table 2. Association between rs7380824 of *STING1* and risk of AMD

Genotypes	Controls	Cases	OR	95% CI	*p*-value	Adjusted*		
						OR	95% CI	*p*-value
CC	72	81	1.0	-	-	1.0	-	-
CT	36	39	0.96	0.55-1.67	0.894	1.17	0.57-2.37	0.664
TT	7	2	0.25	0.05-1.26	0.094	0.20	0.03-1.24	0.084
CC + CT	108	120	1.0	-	-	1.0	-	-
TT	7	2	0.25	0.05-1.26	0.095	0.191	0.03-1.16	0.073

*Adjusted for age, workplace, and smoking habit of the participants

of the innate immune system, plays a significant role in recognizing foreign DNA and inducing inflammatory responses (9). Consequently, the dysregulation of this protein can disrupt the balance between protective and damaging role of immune responses, thereby plays an important role in the pathogenesis of AMD (16). The retina's high metabolic activity and susceptibility to oxidative stress make it prone to chronic inflammation. Therefore, investigating the genetic variations of the *STING1* gene could help explain individual differences in the risk of AMD (17). Based on this rationale, the present study was conducted under the hypothesis that an association exists between the rs7380824 polymorphism and the risk of AMD. However, the study findings did not confirm our hypothesis. Although no significant difference was observed between the genotypes and the risk of developing AMD, the TT genotype, compared to combination of the CC + CT genotypes, had an odds ratio of less than one and was borderline significant (OR = 0.19, 95% CI: 0.03-1.16, $p = 0.073$). The small sample size and low statistical power likely contributed to the lack of a significant association between the genotypes and disease risk. Therefore, investigating this association in larger populations is essential.

In summary, the data of the present study did not provide an association between rs7380824 variant of the *STING1* gene with the risk of AMD in an Iranian population. However, given the different frequency of the genotypes (although nonsignificant) of this gene in AMD patients and healthy subject, investigating this association in a larger population can provide better understanding on the link between this variant and the risk of AMD development.

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Author Contributions

MKL: Genotyping, Analysis and Interpretation, Literature Review and Writing. **MS:** Conceptualization, Design, Supervision, Analysis and Interpretation, Critical Review. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The present study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Shiraz University (IR.US.PSYEDU.REC.1403.038).

Conflicts of Interest

The authors declared that they have no conflict of interest.

Using Artificial Intelligence (AI)

The authors did not use artificial intelligence-based technologies, except for English language corrections using "DeepSeek AI'".

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