Association between genetic polymorphisms of *GPX1* C594T and *GPX4* C718T with the risk of age-related macular degeneration

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ABSTRACT

Purpose: Age-related macular degeneration (AMD) is a progressive disease resulting in loss of vision. One of the important factors in AMD disease is oxidative stress. Excessive accumulation of ROS and the inability of the antioxidant defense system to neutralize it can contribute to the development of lesions. One of the antioxidant enzymes is glutathione peroxidase. The aim of this study was to investigate the relation between genetic polymorphisms of *GPX1* C594T and *GPX4* C718T with the risk of AMD disease.

Materials and Methods: In this case-control study, 122 AMD patients and 122 healthy controls group matched for gender and age. Genotyping of *GPX1* and *GPX4* were done by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method.

Results: There was no significant relationship between allele and genotype frequency in the genetic polymorphisms of *GPX1* C594T (rs1050450) and *GPX4* C718T (rs713041) and susceptibility to AMD. The association between smoking and AMD was found in this study (OR = 2.165, CI = 1.145-4.092, P = 0.017). Also, there was a significant correlation between the number of patients who were working outside the room (OR = 2.067, CI = 1.168-3.659, P = 0.013).

Conclusions: The current study suggested that the *GPX1* C594T (rs1050450) and *GPX4* C718T (rs713041) polymorphisms are not predisposing to AMD.

Keywords: Age related macular degeneration (AMD), *GPX1*, *GPX4*, Polymorphism.

INTRODUCTION

AMD is the disease that was first described in 1875 in medical articles known as: "Diseases That Occur in the Elderly. This disease is caused by the destruction of the retina generally in people over 55 years of age and as the disease progresses it leads to blindness. There are many environmental and genetic factors involved in this disease. These include age, gender, race, diet, smoking, UV light, oxidative stress, and cardiovascular disease.[1-6] The prevalence of AMD is higher in Caucasians than in other races and in women than in men, according to the 2013 NEI report. The accumulation of gradual damage to the retina due to oxidative stress may increase the risk of developing AMD with age.[4,7] In fact, oxidative stress may play an important role in the onset of AMD and in the progression of the disease. Recent studies have shown that excessive increase of free radicals and ROS and inability of antioxidant defense system to decrease them seems to be the main factor in AMD development.[7-9] Accordingly, several classes of antioxidative genes have been studied for their association with AMD, including glutathione peroxidase. Glutathione peroxidase is a family of enzymes with peroxidase activity whose main role is the protection of the organism from oxidative stress. In humans, 5 isoforms of selenium-dependent (GPX1-4,6 selenocysteine) and 3 of non-selenium-dependent (5,7,8 cysteine) have been identified. The glutathione peroxidase 1 gene, located in the 3p 21.3 region, encodes one of the most important intracellular antioxidant enzymes and

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is found in the cytosol, mitochondria and nuclei.[10-12] The pro198Leu polymorphism, which has a functional role and is associated with the substitution of C by T in the exon 2 *GPX1* gene. Indeed, replacing proline by leucine in codon 198 is associated with a decrease in the catalytic activity of the enzyme and a reduced protective role of the enzyme against oxidative stress.[13-15]

Another isoform of glutathione peroxidase is the class 4 enzyme, which is a phosphoglutathione peroxidase and is a monomeric enzyme. The gene encoding this enzyme is located in the chromosomal region 19p 13.3, and this enzyme prevents the formation of peroxide phospholipids in the cell membrane. This enzyme is important in preventing beta cell dysfunction, male infertility, choroidal neovascularization, and photoreceptor cell maturation and survival. A polymorphism of C718T with rs 713041, located near the SECIS element, is one of the known variable regions in 3'UTR. This polymorphism alters the activity of the enzyme by replacing selenocystine and binding protein to 3'UTR.[16,17] Since previous reports have shown that the excessive increase in ROS have an important role in the development of AMD, the aim of this study was to determine the relationship between genetic polymorphisms of antioxidant genes (*GPX1* C594T and *GPX4* C718T) and susceptibility to AMD disease in Iranian population.

MATERIALS and METHODS

Participants

Our case-control study included 122 AMD patients (75 male, 47 female) selected from the Department of Ophthalmology, Khalili Hospital, Shiraz (South Iran) and 122 controls (70 male, 52 female) randomly selected from healthy blood donors. Informed consent and questionnaires were completed by all participants. The ethics committee of Shiraz University approved our study. Iranian population is one of the most heterogeneous populations.[18,19] Therefore, we selected our patients and controls from the same ethnic-religious group (Persian Muslims living in Fars province, southern Iran). Subjects were divided into two groups according to occupation: outdoor (farmers, drivers, etc.) and indoor (housewives, teachers, etc.).

SNP genotyping

The genomic DNA was extracted from the whole blood samples. The restriction fragment length polymorphism (PCR-RFLP) assay was used to determine the genotype for *GPX1* C594T (rs1050450)[13] and *GPX4* C718T (rs713041)[20] polymorphisms. Table 1 summarizes the primer sequences and PCR product size. The thermal profile for *GPX1* included an initial denaturation step of 94°C for 4 minutes, followed by 32 cycles at 94°C (30 seconds), 60°C (30 seconds), and 72°C (30 seconds), and a final extension at 72°C for 5 minutes. *GPX4* included an initial denaturation step of 94°C for 4 minutes, followed by 35 cycles at 95°C (30 seconds), 65°C (30 seconds), and 72°C (30 seconds), and a final extension at 72°C for 7 minutes. PCR products of GPX1 C594T and *GPX4* C718T polymorphisms were digested with restriction enzyme Apa I (5U, 37°C, 3 h) and Sty I (5U, 37°C, 4 h), respectively, as described in Table 1. After digestion, DNA products were analyzed by 3% agarose gel electrophoresis.

Statistical analysis

The observed frequencies of two genotypes were assessed for Hardy-Weinberg equilibrium in the control group of the study. Unconditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for the risk of AMD in association with genetic polymorphisms of *GPX1* C594T and *GPX4* C718T. The CC genotype was used as the reference genotype in all of the analyses. Considering the significant age difference between patients and controls, logistic regression was used in further analyses to calculate ORs and 95% CIs for the different genotypes after adjustment for age. Statistical analysis was performed using SPSS ver. 19 (SPSS Inc., Chicago, IL, USA). A probability of $p < 0.05$ was considered statistically significant.

RESULTS

The genotypic frequencies for the rs1050450 and rs713041 polymorphisms in control and AMD patient groups are shown in Table 2. The genotypic frequencies of the *GPX1* C594T ($\chi2=0.143$, df=1, P>0.05) and the *GPX4* C718T

Table 1: *PCR- RFLP information for GPX1 and GPX4*

Polymorphism	Primer sequence (5´- 3´)	PCR product size	Restriction enzyme	Location of polymorphism
GPX1(rs1050450)	F: GTGTGCCCCTACGCAGGTA R: CACACAGTTCTGCTGACACC	314	*Apa I*	Exon 2
GPX4(rs713041)	F: TTTCTAGCTCCACAAGTGTGTG R: AGATCCAGCAGGCTAATTTGTC	226	*Sty I*	3´UTR

($\chi 2$=0.022, df=1, P>0.05) polymorphisms in healthy controls were found to be similar with the expected values based on the Hardy-Weinberg equilibrium distributions.

The association between smoking and AMD was found in this study (OR=2.165, CI=1.145 - 4.092, p=0.017). The place of work (outdoor) significantly increased the risk of AMD (OR=2.067, CI=1.168-3.659, p=0.013) (data not shown). The CC, CT, and TT genotype frequencies for *GPX1* were 42.5%, 43.4%, and 13.3% in controls and 44.3%, 43.4%, and 12.3% in cases, and for *GPX4* were 19.7%, 49.2%, and 28.7% in controls and 27.0%, 44.3%, and 27.9% in cases, respectively. There was no significant association between the genotypes of *GPX1* C594T (TT vs. CC, OR=0.84, 95%CI=0.36- 1.95, P=0.693) and *GPX4* C718T (TT vs. CC, OR=0.64, 95%CI=0.30- 1.35, P=0.247) and susceptibility to AMD (Table 2).

DISCUSSION

AMD is a progressive disease affecting part of the retina which causes partial or complete loss of vision in one or both eyes in people aged 55 or older.[21] Both environmental factors as well as genetic factors are involved in the development of this disease. It is believed that oxidative stress contributes to the pathogenesis of AMD.[22] In the literature, the association between the antioxidant genes polymorphism (*CAT, SOD1* and *GPX)* and other age-related diseases such as breast cancer,[23] type 2 diabetes,[24] prostate,[25] Alzheimer,[26] cataract[27] has been reported. Some studies have shown that increased expression of *GPX4* strongly protects retina from oxidative damage. These data suggest that gene therapy approaches to augment the activity of GPX4 in the retina and RPE should be considered in patients with AMD.[28]

It was expected that AMD would be associated with SNPs in antioxidant genes because AMD is significantly associated with oxidative stress and oxidative stress scavenged by antioxidant enzymes. It has already been shown that AMD is associated with some genetic variations in genes involved in antioxidant pathways. Previous studies with some antioxidant genes showed that a significant decrease in enzymes SOD, CAT and GPX in AMD patients compared to controls, was indicated. Also, the risk of susceptibility to AMD was significantly higher in patients with AMD who had Pro197Leu C/T genotype of *GPX* (OR = 2.78; 95% CI = 1.78-4.35). The A/C genotype and the C allele frequencies of A/C polymorphism of *SOD1* gene significantly reduce the risk of AMD (OR=0.48; 95% CI 0.27; 0.85).[29]

Therefore, our previous hypothesis was that SNPs of GPX would be associated with susceptibility to AMD. The results of the present study are not in agreement with the above-mentioned previous studies and did not support our hypothesis. There is no definitive conclusion at this time and we will have to wait for further studies from other countries with larger sample sizes.

Table 2: *Association between GPX1 C594T and GPX4 C718T polymorphisms and risk of AMD*

Polymorphism	Case (%)	Control (%)	OR	CI (95%)	P
***GPX1* C594T**					
CC	54(44.3)	51(42.5)	1		
CT	53(43.4)	53(43.4)	1.01	0.57-1.78	0.969
TT	15(12.3)	16(13.3)	0.84	0.36-1.95	0.693
CT+TT vs CC	68(55.7)	69(56.6)	0.93	0.56-1.54	0.782
Alleles					
C	161(65.9)	155(64.5)	1		
T	83(34.1)	85(35.5)	0.94	0.64-1.36	0.746
***GPX4* C718T**					
CC	33(27.0)	24(19.7)	1		
CT	54(44.3)	60(49.2)	0.67	0.34-1.32	0.255
TT	34(27.9)	34(28.7)	0.64	0.30-1.35	0.247
CT+TT vs CC	88(72.1)	94(77.0)	0.70	0.39-1.28	0.258
Alleles					
C	120(49.5)	108(45.4)	1		
T	122(50.5)	130(54.6)	0.84	0.59-1.20	0.356

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