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Glutathione S-Transferase *GSTM 1*, Null Genotype May Be Associated with Susceptibility to Age-Related Cataract

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Abstract

Background. Age-related cataract (ARC) is the leading cause of visual disability and reversible blindness all over the world. The different expressions of GST isozymes among animals may explain the variations in the cataract formation caused by oxidative stress.
Objectives. In this study, we evaluated the distribution of GST gene polymorphisms in ARC patients and the possible associations between the presence of ARC and GST gene polymorphisms.
Material and Methods. The epidemiological data was collected by a standard questionnaire and blood samples were obtained from 130 ARC patients and 159 healthy controls. Data about smoking habits of the groups was recorded. Real-time polymerase chain reaction-based methods were used to detect genetic polymorphisms.
Results. The *GSTM 1* null genotype was found to carry an increased risk for developing ARC (OR: 1.84, 95% CI: 1.13–2.99). The frequency of the *GSTT 1* null genotype was not significantly different among the ARC patients and the controls (OR: 1.0, 95% CI: 0.64–1.6). The *GSTP 1* Val/Val genotype was also not significantly different among the ARC patients and control groups (OR: 1.06, 95% CI: 0.50–2.23). *GSTM 1* null genotype was highly frequent in non-smokers (OR: 3.25, 95% CI: 1.66–6.35) and moderately frequent in smokers (OR: 2.50, 95% CI: 1.28–4.86). Also, carrying the combined genotypes of *GSTM 1* null, *GSTT 1* and *GSTP* 1 105-Val allele was seen to have an increased risk of developing ARC (OR: 2.91, 95% CI: 1.31–6.44).
Conclusions. This data may provide evidence that *GSTM 1* gene polymorphisms may be associated with genetic susceptibility to develop ARC. Larger studies are warranted to verify these findings (**Adv Clin Exp Med 2015, 24, 1, 113–119**).

Key words: age-related cataract, glutathione S-transferase, *GSTM 1, GSTT 1, GSTP 1,* polymorphism.

Age-related cataract is one of the leading causes of visual impairment and reversible blindness among older people all over the world [1]. ARC is a complex disease with a broad spectrum of risk factors including age, sex, smoking, exposure to sunlight, estrogen sufficiency or deficiency and cardiovascular factors. Genetic backgrounds also have an important effect on the pathogenesis of ARC [2]. Oxidative stress or damage as a result of increased generation of active oxygen species and free radicals in the lens have been implicated in the aetiopathogenesis of ARC [3]. Free radicals initiate lipid peroxidation leading to increased production of lipid peroxides in various forms of cataract. Oxidative damage can result in several molecular changes, such as degradation, cross-linking, and aggregation of lens proteins that contribute to the development of cataracts. [4, 5].

The eye must possess efficient reducing and detoxification systems, such as superoxide dismutase, catalase, glutathione S-transferase (GST) and glutathione peroxidase for protecting the eye from oxidative damage [6, 7]. It has been hypothesized that protein S-thiolation is the earlier damage to lens proteins during ARC. This may give rise to a cascade of events starting with enzyme deactivation, alteration of protein conformation, protein–protein aggregation, and eventually opacification of the lens [8]. The different expressions of GST isozymes among animals may explain the variations in the cataract formation caused by oxidative stress [9].

The glutathione S-transferases (GSTs; EC: 2.5.1.18) are a superfamily of proteins that participate in phase II of cellular detoxification that catalyze the conjugation of reduced glutathione (GSH) with a variety of electrophilic compounds, thereby protecting the cell against xenobiotics and oxidative stress [10]. Human GST enzymes include cytosolic classes of GST such as α (GSTA), μ (GSTM), π (GSTP), κ (GSTK), θ (GSTT), and ζ (GSTZ), and one microsomal form (mGST, microsomal) [11]. Among these classes of GST; *GSTT 1*, *GSTM 1*, GSTM 3, *GSTP 1*, and GSTZ 1 have been shown to be polymorphically distributed [12, 13]. The member of the GST μ class (*GSTM 1*) and GST θ class (*GSTT 1*) genes both exhibit deletion polymorphisms. Homozygous deletions of those genes, called *GSTM 1* and *GSTT 1* null genotypes, result in a lack of enzyme activity. The *GSTM 1* and GSTT 1 null genotypes have been linked with an increased risk of several multifactorial diseases. GSTM 1 and *GSTT 1* have also been associated with risk of ophthalmologic problems such as ARC [14–16]. Three common polymorphisms in the *GSTP 1*, *GSTT 1*, and *GSTM 1* genes either decrease or suppress the GST enzyme activity. A single nucleotide substitution (A→G) at position 313 of the *GSTP 1* gene, which results in replacing isoleucine with valine, substantially decreases *GSTP 1* enzyme activity [17]. Furthermore, inherited homozygous deletions of the *GSTT 1* or *GSTM 1* genes lead to an absence of enzymatic activity [18].

Recent studies have linked cigarette smoking to an increased risk of developing cataracts in men and women [19]. Several studies have indicated that GST enzymes may play a role in cataract predisposition with different GST phenotypes [16, 20–23] but no associations were found in another study [24].

In the present study, we aim to determine the polymorphic variants of *GSTM 1*, *GSTT 1*, *GSTP 1* genotypes of smoking status in healthy individuals and ARC patients from the Mersin region of Turkey. We also aim to investigate the possible associations between developing ARC and GST *(GSTM 1*, *GSTT 1*, and *GSTP 1)* polymorphisms.

Material and Methods

Polymorphisms of groups of subjects were compared; there were 130 ARC patients, and 159 controls. Patients with ARC were recruited from the Ophthalmology Department of the University Hospital, Mersin. The control subjects were collected from volunteers living in the same region. ARC patients and controls were unrelated. Both ARC patients and control groups were interviewed about smoking habits, and classified as non-smokers and smokers. At the time of blood donation, each individual completed a questionnaire outlining their personal history of several disorders. The current study was approved by the Ethics Committee of Mersin University, Turkey. The study population was informed about the objectives of the study and consent was received for their participation. All subjects with hypertension, thyroid function disorders, diabetes mellitus, anemia, renal and liver dysfunction, inflammatory arthritis and osteoporosis were excluded from this study. Patients with ARC formation secondary to identifiable causes, such as steroid administration, trauma and diabetes were also excluded. After enrollment, all subjects were undertaken a detailed lens examination to determine the final cataract status. During the examination, Lens Opacities Classification System II (described by Chylack et al.) which uses photographic standarts for grading cataract type and severity was used to grade cataract status with slitlamp examination [25].

Analysis of *GSTM 1, GSTT 1* and *GSTP 1* Polymorphism

Genomic DNA was extracted from 200 μL of peripheral blood by High Pure DNA isolation Kit (Qiagen, Inc., Chatsworth, CA) following manufacturer instructions. Real time – polymerase chain reaction (RT-PCR) was used to detection of *GSTT 1, GSTM 1* and *GSTP 1* gene polymorphisms. For *GSTM 1* polymorphism 5'-GAA CTC CCT GAA AAG CTA AAG C-3'and 5'-GTT GGG CTC AAA TAT ACG GTG G-3' primers and 5'--LCR 640- ATG GCC GCT TCC CAG AAA CTC TG-3' and 5'-TCA CTC CTC CTT TAC CTT GTT TCC TGC AAA -FL-3' hybridization probes were used. For GSTT 1 polymorphism 5'--TTC CTT ACT GGT CCT CAC ATC TC-3' and 5'-TCC AGG TCA ACC GGA TCA T-3' primers 5'-LCR 640- TCD AAG GCC GAC CCA AGC

TGG C-3' and 5'-CCG TGG GTG CTG GCT GCC AAG T-FL-3' hybridization probes was used. For GSTP 1 polymorphism 5'-ACC CCA GGG CTC TAT GGG AA-3' and 5'-TGA GGG CAC AAG AAG CCC CT-3'primers and 5'LCR 640- TGT GAG CAT CTG CAC CAA GGG TTG GGG-3' and 5'-TGC AAA TAC ATC TCC CTC ATC TAC ACA AC-FL-3' hybridization probes were used. The PCR primers were synthesized according to Ko et al. [26]. In order to avoid false negative readings, ß-globin gene was used as internal control. Also, negative control was used in all run to test contamination.

Statistical Analysis

In order to compare the ages of two groups, the student's *t* test was used. A case-control study was performed and allelic frequency of the polymorphism was calculated both in cases and controls. The χ^2 test was used to compare genotype frequency of the *GSTM 1, GSTT 1* and *GSTP 1* gene polymorphisms between patients with ARC and controls. The association between *GSTT 1, GSTM 1* and *GSTP 1* polymorphisms, smoking, and ARC and DM were modeled through binary multivariate regression analysis and odds ratio (OR) and 95% confidence interval (95% CI) were calculated to compare ARC risk around genotypes. P value less than 0.05 was considered as significantly different. The software used for the calculation was the SPSS version 11.5 (SPSS Inc., Chicago, IL).

Results

In this study, we used a PCR based genotyping assay to examine a polymorphism from each of three GST genes, *GSTM 1, GSTP 1* and *GSTT 1* in ARC susceptibility. Table 1 describes the distribution of the ARC patients, and the controls by sex, age, and smoking status as appropriate. For smoking status, the data was suggestive of a trend of increasing risk for higher numbers of smokers (compared to non-smokers); showing a 1.3-fold increased risk of ARC patients (95% CI: 0.82–2.13) but this increase was not significant. The genotypic results for each gene can be seen in Table 1. The frequency of the *GSTM 1* null genotype in ARC patients (56.9%) was higher than in controls (40.9%), showing an increased risk of developing ARC (OR: 1.84, 95% CI: 1.13–2.99). The frequency of the *GSTT 1* null genotype was not significantly different between the ARC patients and the controls (OR: 1.0, 95% CI: 0.64–1.6). The *GSTP 1* Val/Val genotype was also not significantly different between the ARC patients and the control groups (OR: 1.06, 95% CI: 0.50–2.23). The variant genotype, termed Val/Val, was seen in 12.3% of the ARC patients and in 15.7% of the controls. The

Table 1. Comparison of patients with ARC and control group and GST genotypes and the risk of developing ARC

Variable	ARC (n = 130)	Controls (n = 159)	‡OR (95% CI)
Age, years mean ± SD	66.41 ± 6.8	62.74 ± 6.5	1.08 (1.04–1.13)
Sex standard deviation			0.90 (0.54–1.49)
female	56 (43.1)	69 (43.4)	
male	74 (56.9)	90 (56.6)	
Smoking status			
non-smokers	63 (48.5)	90 (56.6)	1 (reference)
smokers	67 (51.5)	69 (43.4)	1.37 (0.82–2.13)
GSTM 1#			
present	54 (41.5)	94 (59.1)	1 (reference)
null	76 (56.9)	65 (40.9)	1.94 (1.16–3.25)
GSTT 1#			
present	101 (77.7)	118 (74.2)	1 (reference)
null	29 (22.3)	41 (25.8)	0.80 (0.44–1.44)
GSTP 1			
Ile/Ile	54 (41.5)	76 (47.8)	1 (reference)
Ile/Val	60 (46.2)	58 (36.5)	1.62 (0.95–2.78)
Val/Val	16 (12.3)	25 (15.7)	1.16 (0.53–2.51)

n – number of observation; ‡ ORs (odds ratio), CI (confidence interval) from multiple binary logistic regression.

carriers of at least one intact allele are used as reference.

Table 2. Odds ratios for ARC with smoking status combinations of GSTs genotypes*

Variable	Smoking status	ARC n (%)	Control n (%)	‡OR (95% CI)
GSTM 1 present**	nonsmokers	24 (18.5)	60 (37.7)	1 (reference)
	smokers	32 (24.6)	34 (21.4)	2.35 (1.19–4.62)
GSTM 1 null	nonsmokers	39 (30.0)	30 (18.9)	3.25 (1.66–6.35)
	smokers	35 (26.9)	35 (22.0)	2.50 (1.28–4.86)
GSTT 1 present**	nonsmokers	54 (41.5)	65 (40.9)	1 (reference)
	smokers	52 (40.0)	53 (33.3)	0.44 (0.15–1.27)
GSTT 1 null	nonsmokers	9 (6.9)	25 (15.7)	1.02 (0.45–2.31)
	smokers	15 (11.5)	16 (10.1)	1.25 (0.55–2.85)
GSTP 1 Ile/Ile	nonsmokers	28 (21.5)	41 (25.8)	1 (reference)
	smokers	26 (20.0)	36 (22.6)	1.05 (0.52–2.12)
GSTP 1 Ile/Val or Val/Val	nonsmokers	35 (26.9)	49 (30.8)	1.04 (0.54–1.99)
	smokers	41 (31.5)	33 (20.8)	1.81 (0.93–3.53)

* GSTs – glutathione S-transferase M 1, T 1, and P 1 genes; n – number of observation; ‡ ORs (odds ratio); CI (confidence interval) from multiple binary logistic regression; ** carriers of at least one intact allele are used as reference.

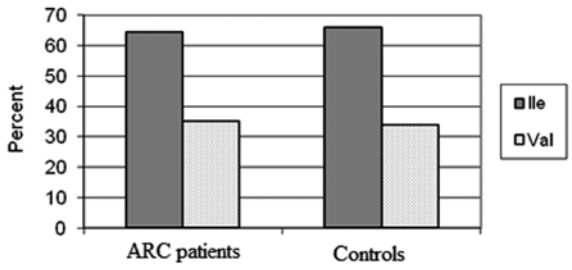

Fig. 1. Percent of GSTP1 allele frequences among ARC patients and controls

frequency of *GSTP 1* Val-105 allele was 0.34 for controls and 0.35 for ARC patients. There was no significant difference between ARC patients and controls in the frequency of these alleles (p = 0.72) (Fig. 1).

The effect of smoking status on the risk of developing ARC has varied according to the genotypes (Table 2). For cases with the *GSTM 1* present genotype, there was a slightly higher risk associated with current smoking habits (OR: 2.35, 95% CI: 1.19–4.62). We have found higher risk for nonsmokers (OR: 3.25, 95% CI: 1.66–6.35) and moderate risk for smokers (OR: 2.50, 95% CI: 1.28–4.86) among cases with *GSTM 1* null genotype. We have also not observed a risk among *GSTT 1* null and *GSTP 1* Val/Val genotypes in cases of smokers.

To investigate whether the profiles of *GST* genotypes were associated with the risk of developing ARC or not, we have examined the combinations of genotypes.

Table 3 displays the risk of developing ARC associated with each combination of genotypes. The combined *GSTM 1* null, *GSTT 1* present and *GSTP 1* 105-Val allele genotypes seem to have an increased risk of developing ARC (OR: 2.91, 95% CI: 1.31–6.44). These results indicate that *GSTM 1* gene may have an influence in developing ARC in this study.

Table 3. Association between GST genotype profile and the development of ARC

GSTM 1	*GSTT 1*	*GSTP 1*	ARC n (%)	Control n (%)	OR (95% CI)
1. Present	present	Ile/Ile	16 (12.3)	29 (18.2)	1 (reference)
2. Null	present	Ile/Ile	28 (21.5)	33 (20.8)	1.36 (0.63–2.96)
3. Present	null	Ile/Ile	5 (3.8)	7 (4.4)	0.92 (0.23–3.59)
4. Present	present	Ile/Val or Val/Val	20 (15.4)	35 (22.0)	1.10 (0.50–2.42)
5. Null	null	Ile/Ile	5 (3.8)	8 (5.0)	0.80 (0.21–3.06)
6. Null	present	Ile/Val or Val/Val	37 (28.5)	21 (16.2)	2.91 (1.31–6.44)
7. Present	null	Ile/Val or Val/Val	13 (10.0)	25 (13.2)	0.70 (0.27–1.80)
8. Null	null	Ile/Val or Val/Val	6 (4.6)	3 (1.9)	2.14 (0.43–10.72)

Discussion

Age related cataract is a vision impairing disease. The pathophysiology behind ARC is complex and not yet fully understood [22]. According to the reports given by the world health organization, 18 million people suffer from blindness caused by ARC [1]. Epidemiologic studies have shown that cataract is associated with many environmental factors such as ultraviolet-B exposure, smoking, alcohol consumption, and use of steroids. Recently, genetic factors have been found to play important roles in the development of ARC [2].

It is believed that oxidation is a very early or initiating event in the overall process of events leading to cataract formation [3, 5]. Oxidative stress has been suggested to be a common underlying mechanism of cataractogenesis, and augmentation of the antioxidant defenses of the ocular lens has been shown to prevent or delay its occurrence. Oxidation-reduction mechanisms have special importance in the eye. Oxidative damage has important role in the development of glaucoma, cataract and other eye diseases by resulting in a number of molecular changes in the eye [3, 5, 6, 27]. The peroxidative damage to the lens cell membranes and biomolecules, induced by the lack of reductive detoxification of phospholipid hydroperoxides, has been proposed as a triggering mechanism of cataractogenesis. One of the major events in the pathogenesis of cataract is this peroxidative damage might be mediated by toxic metabolites of oxygen such as OH, H_2O_2, and O_2 as a result of the reduced enzymatic defenses against their toxicity [28]. The GSTs are phase II enzymes that play critical roles in protection against products of oxidative stress and electrophiles. The GST enzymes conjugate hydrophobic and electrophilic compounds with reduced glutathione. Genetic polymorphisms have been reported for *GSTM 1, GSTP 1* and *GSTT 1*, resulting in either decreased or altered enzyme activity. Polymorphisms in genes coding for enzymes involved in the protection against oxidative stress have been implicated in the predisposition of individuals to disease states such as cancer. Glutathione and glutathione-dependent enzymes represent a coordinately regulated defense against oxidative stress [29, 31].

A study performed by Alberti et al. [24] reported that *GSTM 1* null genotype was found to be in similar frequencies in both the ARC patients and the control group (51% and 50%, respectively). In contrast, Sekine et al. [16] have demonstrated a significant inverse association between *GSTM 1* null genotype and cataract development in elderly patients (OR: 2.91, 95% CI: 1.56–5.44). Also, Juronen et al. [20] designed a study in a patient group having senile cortical cataracts, and examined the polymorphisms of *GSTM 1, GSTM 3, GSTT 1*, and *GSTP 1*. They have found a two-fold risk of developing senile cortical cataracts associated with the *GSTM 1*-positive phenotype. Similarly Chandra et al. [21] also found 1.9 (95% Cl: 1.08–3.32) risk of developing cataract with the *GSTM 1*-positive phenotype in Indian population. However, *GSTT 1*-positive phenotype has protective effect (OR: 0.27, 95% CI, 0.09–0.86) was determined in this study.

Another study was carried out to examine the relationship between *GSTM 1*, *GSTT 1* gene polymorphisms and cortical ARC in the Han Chinese population. And they showed that the *GSTM 1* positive genotype had an increased risk of developing cortical ARC ($p = 0.0002$, odds ratio [OR] 1.74, 95% CI 1.30 to 2.34). Also, it is found that there is a relationship between a combination of *GSTM 1* positive and *GSTT 1* null genotypes and the risk of developing cortical ARC ($p = 0.002$, OR 2.19, 95% CI 1.33 to 3.60). But no association was found between *GSTT 1* genotypes and cortical ARC [23]. On the other hand, Sireesha et al. who investigated the relationship between *GSTM 1* and *GSTT 1* gene polymorphisms and ARC, found that *GSTM 1* positive, *GSTT 1* null and double null (*GSTM 1* null and *GSTT 1* null) genotypes may confer risk for the development of ARC [22].

Another variant investigated in this study was *GSTP 1*, where the carriers of the *GSTP 1**A allele conducted a three-fold higher risk of developing senile cortical cataract in Juronen et al.'s study. Also, the risk of developing senile cortical cataract was highest in people having all three predisposing genotypes: *GSTT 1*-positive, *GSTM 1*-positive, and *GSTM 3* AA.

In our study, we have found a significant difference between frequencies of the *GSTM 1* null genotype in the tested populations. The positive genotype was found to have a protective effect against the development of ARC, but *GSTT 1* and *GSTP 1* polymorphisms have not been effective in ARC development. Jiang et al. [23] and Juronen et al. [20] found that *GSTT 1* null genotype was not associated with senile cataract development. Similarly, we have showed that in the ARC patients *GSTT 1* null genotype was not significantly different from the control group. The frequencies of two alleles (Ile-105 and Val-105) of *GSTP 1* locus and *GSTP 1* genotypes were found to be different in senile cortical cataract patients in Juronen's study. Unlike Juronen, we have found no difference in ARC patients and controls for the prevalence of *GSTP 1* alleles and genotypes.

This inconsistency in reports may be attributed to a number of factors, including ethnic variability in *GSTM 1* and *GSTT 1* null genotype distribution, which ranges between 10% to 88% in different ethnic populations [31] and to the types of environmental factors involved in the pathogenesis of cataract. The recent meta-analysis by Sun et al. [32] reported the association between *GSTM 1* and *GSTT 1* null genotypes and the risk for senile cataract which was statistically significant in Asians, but not in Caucasians.

Juronen et al. [20] investigated the association between GST polymorphism, smoking status, and senile cataract incidence in the cataract patients. This study showed that in the *GSTM 1*-positive phenotype, the susceptibility to senile cataract development was weekly associated in smokers when compared with non-smokers, but this relation was not statistically significant (OR: 1.63; 95% CI: 0.79–3.67). Our study has demonstrated possible associations between *GSTM 1* null genotype and age-related cataract, also interactions between *GSTM 1* null genotype and smoking status. Also, we have found an additive effect of the *GSTM 1* null, *GSTT 1* present, and *GSTP 1* 105-val allele genotypes on genetic predisposition for developing ARC caused by *GSTM 1* null genotype.

Polymorphisms of this gene result in either the production of an enzyme known to play a role in Phase II detoxification of polycyclic aromatic hydrocarbons found in tobacco smoke or no production of enzyme (deletion polymorphism). Tobacco smoke is considered a risk factor in larynx cancer, and the *GSTM 1* null genotype was associated with an increased risk in this disease, but *GSTM 1* detoxifies reactive metabolites of benzo *a* pyrene, other PAH, and aflatoxin B 1 only among smokers [33, 34].

In conclusion, ARC patients with *GSTTM 1*-null genotype may have an increased risk of developing cataract. The present study had limited statistical power for evaluation of gene-environment interactions due to the relatively small number of ARC patients. However, the direction of the associations is toward to biologically plausible increased risks. The combination of high well-done tobacco consumption with *GSTM 1* as a susceptible genotype should be evaluated in larger studies.

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