European Journal of Ophthalmology / Vol. 16 no. 1, 2006 / pp. 105-110

Glutathione S-transferase M1, T1, and P1 gene polymorphism in exudative age-related macular degeneration: A preliminary report

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PURPOSE. *To elucidate whether the gene polymorphisms of glutathione S-transferase (GST) M1, T1, and P1 are associated with the development of exudative age-related macular degeneration.*
METHODS. *The authors genotyped 35 white patients with exudative age-related macular degeneration and 159 healthy controls. Genomic DNA from peripheral blood was examined using polymerase chain reaction and defined for the genetic polymorphisms of GST.*
RESULTS. *No association was observed between GSTM1, GSTT1, and GSTP1 polymorphisms and age-related macular degeneration risk (p>0.05). The frequencies of the combination of the GSTM1 (null) and GSTP1 (mutant), GSTM1 (null), and GSTT1 (null) genotype polymorphisms in patients with exudative age-related macular degeneration differed greatly from those of the control group (p=0.001 OR [95% CI]: 7.70 [2.28–25.98] and p=0.007 OR [95% CI]: 3.88 [1.51–10.02], respectively).*
CONCLUSIONS. *The present study suggests that the GSTM1 (null) and GSTT1 (null), GSTM1 (null), and GSTP1 (mutant) combinations may be a genetic risk factor for the development of exudative age-related macular degeneration. However, the potential role of GST polymorphisms as a marker of susceptibility to age-related macular degeneration needs further studies in a larger number of patients. (Eur J Ophthalmol 2006; 16: 105-10)*

KEY WORDS. *Age-related macular degeneration, Oxidative stress, Glutathione S-transferase, Gene polymorphism*

Accepted: August 31, 2005

INTRODUCTION

Age-related macular degeneration (ARMD) is the leading cause of severe visual loss and a major cause of irreversible blindness in developed countries (1-3). The prevalence and severity of ARMD increase substantially with age. Although approximately 10% of patients with ARMD manifest the exudative (neovascular) form of the disease, the vast majority of people with severe vision loss (20/200 or worse in either eye) from ARMD have the exudative form (4).

ARMD is considered a multifactorial disease caused by an interaction between the environment and multiple gene loci (5). The degree of heritability and the relative role of genetic and environmental factors are unknown. This is currently under investigation. Epidemiologic studies have demonstrated that genetic, systemic, behavioral, and lifestyle factors, such as smoking status, nutrition, and sun-

 1120-6721/105-06$15.00/0

light exposure, are known risk factors for ARMD (4, 6-16).

There is a general consensus that cumulative oxidative damage is responsible for aging, and may therefore play an important role in the pathogenesis of ARMD (17). Oxidative stress and antioxidant systems are potentially important for ocular tissues. To understand the multifactorial causes of ARMD, it is reasonable to investigate whether genetic polymorphisms of antioxidant enzymes contribute to the development of ARMD. Several epidemiologic studies suggest that individual susceptibility to several disorders might be connected with the glutathione S-transferase (GST) system (18, 19). GSTs are a group of enzymes known to play an important role in the detoxification of several endogenous and exogenous toxic and carcinogenic substances. Most harmful components found in tobacco smoke are activated by phase I enzymes such as cytochrome p450 1A1 and become a carcinogen in the form of reactive episodes. Then these carcinogens are detoxified by several phase II enzymes such as GSTs (20). The GST isoenzymes expressed in human tissue comprise Alpha (A), Mu (M), Pi (P), Theta (T), Kappa (K), Omega (O), and Zeta (Z) gene families. Among these classes of GST, GSTM1, GSTM3, GSTT1, GSTP1, and GSTZ1 have been shown to be polymorphically distributed (21-25).

In this study, we investigated the distribution of GSTM1, GSTT1, and GSTP1 polymorphism in patients with exudative ARMD and controls to explore the possible association between different GST variants and the development of exudative ARMD.

MATERIALS AND METHODS

This study was a hospital-based case-control study conducted at the Ophthalmology Department of Mersin University Hospital during the period of 2002–2004. Control subjects (n=159) and patients with exudative ARMD (n=35) were consecutively selected with no history of cardiovascular disease, cancer, chronic degenerative neurologic disease, chronic obstructive pulmonary disease, hepatitis, allergies in general, alcohol abuse, or smoking. The patients and controls were from the same geographic region and of the same ethnic origin. Cases and controls were unrelated.

Ophthalmologic examination was performed with measurement of visual acuity, slit lamp, measurement of intraocular pressure, and ophthalmoscopy. Control and patients with exudative ARMD had no others ophthalmic diseases, such as glaucoma or visually significant cataract. The type of ARMD was the exudative form, defined by ophthalmoscopic and fluorescein angiographic findings including choroidal neovascularization (CNV) and associated manifestations such as serous or hemorrhagic detachment of the retinal pigment epithelium or sensory retina, subretinal hemorrhage, and fibrovascular disciform scarring. Control individuals were enrolled for the far and/or near vision testing and they were not followed up. There was no evidence of ARMD by fundus examination in the control group. Patients were treated, if needed, with diode laser photocoagulation or photodynamic therapy

TABLE I - POLYMERASE CHAIN REACTION (PCR) PRIMER SEQUENCES AND HYBRIDIZATION PROBES FOR GLUTATHIONE S-TRANSFERASE (GST) M1, T1, AND P1

Gene	PCR primers	Hybridization probes
GSTM1	5'-GAACTCCCTGAAAAGCTAAAGC-3'	5'-LCR640-ATGGCCGCTTCCCAGAAACTCTG-3'
	5'-GTTGGGCTCAAATATACGGTGG-3'	5'-TCACTCCTCCTTTACCTTGTTTCCTGCAAA-FL-3'
GSTT1	5- TTCCTTACTGGTCCTCACATCTC-3'	5'-LCR640-TCDAAGGCCGACCCAAGCTGGC-3'v
	5'-TCCAGGTCAACCGGATCAT-3'	5'-CCGTGGGTGCTGGCTGCCAAGT-FL-3'
GSTP1	5'-ACCCCAGGGCTCTATGGGAA-3'	5'LCR640-TGTGAGCATCTGCACCAAGGGTTGGGG-3'
	5'-TGAGGGCACAAGAAGCCCCT-3'	5'-TGCAAATACATCTCCCTCATCTACACAAC-FL-3'
ß-globin	5'-CAACTTCATCCACGTTCACC-3'	5'-GAAGAGCCAAGGACAGGTAC-3'

after the diagnosis of ARMD, and controlled at least one time every 3 months with full ophthalmologic examinations including fluorescein angiography.

The patient group consisted of 35 white patients with exudative ARMD (16 male, mean age 66±7.6 years, range 59–74; 19 female, mean age 63±8.1 years, range 57–72). We recruited 159 individuals as control subjects (90 male, mean age 65±6.7, range 59–72; 69 female, mean age 62±8.6, range 56–64). Informed consent was obtained from all subjects after explanation of the nature of the study.

Blood samples and DNA isolation

Venous blood was collected via venapuncture in sterile siliconized EDTA 2-mL Vacutainer tubes. Immediately after collection, whole blood was stored at +4 °C until use. Genomic DNA was extracted from whole blood using High Pure PCR Template Preparation kits (Roche Diagnostics, GmbH, Germany).

An analysis of GSTM1, GSTT1, and GSTP1 polymorphism

The detection of GSTT1, GSTM1, and GSTP1 gene polymorphisms were made by using Real-Time PCR (Roche Diagnostics, GmbH, Mannheim, Germany).

Appropriate fragment of the GST gene for GSTT1, GSTP1, and GSTM1 was amplified with specific primers from human genomic DNA. The nature of mutations is gene deletions for GSTT1 and GSTM1, and single nucleotide substitution (A→G) at position 313 of GSTP1 gene. The PCR primers were synthesized according to the Ko Y (26). The sequences and the hybridization probes are shown in Table I.

To test GSTM1 and GSTT1 false negative subjects, a simple polymerase chain reaction (PCR) was used to identify nulled subjects. The PCR was performed in a Techne thermal cycler (Progene). ß-globin gene was co-amplified as an internal positive control in the individual PCR reactions. Both the PCR primers and hybridization probes were synthesized by TIB MOLBIOL (Berlin, Germany).

Statistical analysis

The statistical program SPSS 11.5 for Windows was used. Student's t test was used for comparing the ages of two groups. Chi-square test was used for comparing the two independent proportions. All values are represented as mean and standard deviation (SD). p Values of <0.05 were considered statistically significant.

GSTP1 genotype frequency distributions observed were compared with expected from the Hardy-Weinberg equation and analyzed by chi-square test.

The association between GSTM1, GSTT1, and GSTP1 polymorphisms and exudative ARMD disease was modeled through multivariate logistic regression analysis.

TABLE II - GST GENOTYPES AND THE RISK OF DEVELOPING EXUDATIVE AGE-RELATED MACULAR DEGENERATION

Variable*	Cases (n=35) n (%)	Controls (n=159) n (%)	p†	OR (95% CI)‡
GSTM1				
Present	18 (51.4	96 (60.4)	0.333	1 (reference)
Null	17 (48.6)	63 (39.6)		1.44 (0.69–3.00)
GSTT1				
Present	25 (71.4)	118 (74.2)	0.736	1 (reference)
Null	10 (28.6)	41 (25.8)		1.15 (0.51–2.58)
GSTP1§			0.826	
Ile/Ile	16 (45.7)	76 (47.8)		1 (reference)
Ile/Val	12 (34.3)	58 (36.5)		0.98 (0.43–2.24)
Val/Val	7 (20.0)	25 (15.7)		1.33 (0.49–3.60)

*Carriers of at least one intact allele (present) or GSTP1 Ile/Ile genotype are used as reference
†Chi-square test result
‡Matched odds ratio (OR), confidence interval (CI) from conditional logistic regression
§GSTP1 genotype frequency distribution is in the Hardy-Weinberg equilibrium (p=0.13 in patients; p=0.07 in controls)

Odds ratio and confidence intervals were used to analyze the occurrence frequencies of GSTM1, GSTT1, and GSTP1 genotypes in patients with exudative ARMD compared to the control groups.

The GST assays place individuals into distinct categories: those with present or null genotypes for GSTM1 and GSTT1 and homozygous 105 Ile, heterozygous or homozygous 105 Val genotypes for GSTP1. The reference group consisted of individuals with three putative low-risk genotypes, i.e., the presence of GSTM1 (non-deleted), GSTT1 (non-deleted), and GSTP1 (homozygous Ile-105) functional alleles.

RESULTS

There was no difference between ages of control and exudative ARMD patients (p=0.864).

The observed GSTP1 genotype frequencies in the patients (p=0.13) as well as controls (p=0.07) were in Hardy-Weinberg equilibrium.

No association was observed between GSTM1 (p=0.333), GSTT1 (p=0.736), and GSTP1 (p=0.826) polymorphisms and exudative ARMD risk (Tab. II).

We analyzed the genotypes of GSTM1/GSTT1, GSTM1/GSTP1, and GSTT1/GSTP1 in combination to evaluate whether combinations of these genotypes are associated with the development of exudative ARMD. In these combinations, we accepted GSTP1 gene as functional that had at least one intact Ile allele and nonfunctional (mutant) that had val/val genotype. The frequencies of the combination of the GSTM1 (null) and GSTT1 (null), GSTM1 (null), and GSTP1 (mutant) genotype polymorphisms in patients with exudative ARMD differed greatly from those of the control group ($p<0.05$; Tab. III). The risk of exudative ARMD was increased 3.88-fold in both null GSTM1 and GSTT1 gene locus combination and 7.70-fold in null GSTM1 and mutant GSTP1 gene locus combination.

DISCUSSION

The exudative form of ARMD, characterized by CNV, is responsible for most cases of severe visual loss. The etiology of ARMD is largely unknown, but undoubtedly environmental and genetic factors have been implicated in the disease. Retina is particularly susceptible to oxidative stress because of its exposure to visible light and its high consumption of oxygen (17). There is general consensus that oxidative tissue damage plays a causative and contributing role in the pathogenesis of ARMD (17, 27). It is reasonable to study whether genetic polymorphisms of antioxidant enzymes contribute to the development of ARMD.

Glutathione is the key component of a ubiquitous antioxidant system that defends the cell against the toxic effects of reactive oxygen species. Exposure to light by

TABLE III - DISTRIBUTION OF GST GENOTYPES IN COMBINATIONS AND THE RISK OF DEVELOPING EXUDATIVE AGE-RELATED MACULAR DEGENERATION

Combinations of the variables*	Cases (n=35) n (%)	Controls (n=159) n (%)	p†	OR (95% CI)‡
GSTM1/GSTT1			0.007	
Present	26 (74.3)	146 (91.8)		1 (reference)
Null	9 (25.7)	13 (8.2)		3.88 (1.51–10.02)
GSTM1/GSTP1				
Present/Functional	28 (80.0)	154 (96.9)		1 (reference)
Null/Mutant	7 (20.0)	5 (3.1) 0.001		7.70 (2.28–25.98)
GSTT1/GSTP1			0.545	
Present/Functional	28 (80.0)	134 (84.3)		1 (reference)
Null/Mutant	7 (20.0)	25 (15.7)		1.34 (0.53–3.40)

*Carriers of at least one intact allele (present) and at least one Ile allele (functional) are used as reference
†Chi-square test result
‡Matched odds ratio (OR), confidence interval (CI) from conditional logistic regression

photosensitizing mechanisms may lead to the formation of reactive oxygen species. Glutathione detoxifies a variety of exogenous and endogenous substances both nonenzymatically and via enzymatic conjugation catalyzed by GSTs (28). GSTs catalyzed the conjugation of reduced glutathione with a wide variety of electrophiles that include known carcinogens and various compounds that are products of oxidative stress, including oxidized DNA and lipid. Indeed, several lines of evidence suggest that the level of expression of GST is a crucial factor in determining the sensitivity of cells to a broad spectrum of toxic chemicals and oxidative stress (21, 22).

Homozygous deletion variants in GSTM1 and GSTT1 genes (null genotypes) have been found useful for molecular cancer studies. The null genotype frequency in GSTM1 varies from 38% to 67% in whites (29). Null genotype frequency in GST T1 is approximately 20% in whites (29). To our knowledge, association between exudative ARMD and GST polymorphism were investigated in only one study. In this study, Kimura et al (30) studied five genetically polymorphic enzymes, cytochrome P-450 1A1, GSTs (GSTT1 and GSTM1), microsomal epoxide hydrolase, and manganese superoxide dismutase, that play roles in protection against environmental insult and are conceivably related to susceptibility to exudative ARMD. They found significant association of manganese superoxide dismutase gene polymorphism and a weak association of microsomal epoxide hydrolase gene polymorphism with ARMD.

Nevertheless, genotype frequency of cytochrome P-450 1A1 and GSTs (GSTT1 and GSTM1) did not show a statistically significant difference, but they did not analyze risk of the GST gene polymorphism combinations and did not show the data in the article. However, they clarified that GSTM1 and GSTT1 frequency distributions are similar in Japanese and white control populations. Similarly, we did not find any association between GSTM1, GSTT1, and GSTP1 polymorphisms and exudative ARMD risk. The frequencies of the GSTM1 null genotype and GSTP1 mutant genotype combination and the null genotypes of both GSTM1 and GSTT1 in patients with exudative ARMD differed greatly from those of the control group. To our knowledge, this preliminary report is the first study in the literature that demonstrates that GST gene polymorphisms may act as a genetic risk factor for ARMD in various combinations. Despite our small sample size, our results indicate that individuals with GSTM1 (null) and GSTP1 (mutant) are at higher relative risk to develop exudative ARMD than individuals with the alternate combination. This may be attributed to the difference in the type of environmental factors involved in exudative ARMD in a different population. Both the GSTM1 (null) and GSTP1 (mutant) homozygotes have been of particular interest because individuals with these genotypes may demonstrate impaired ability to detoxify xenobiotic and endogenous substrates, and therefore have an increased risk of various disease developments (31).

In conclusion, combination of GSTM1 (null) and GSTP1 (mutant) genotypes and combination of GSTT1 and GSTM1 null genotypes result in a prominent risk for the development of ARMD. However, further studies using a larger sample size are needed to define the association between GSTs and exudative ARMD.

The authors have no commercial or financial incentive associated with publishing this article.

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