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SHORT COMMUNICATION

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Glutathione S-transferase polymorphisms in patients with Behçet's disease

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The glutathione S-transferases (GST) are a multigene family of enzymes involved in the detoxification and, in a few instances, activation of a wide variety of chemicals. GST might contribute to protection against oxidative stress, either by direct inactivation of peroxidized lipids and DNA or by detoxification of xenobiotics, which are known cofactors for radical formation. The GST isoenzymes expressed in human tissue comprise the 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 [1, 2].

Behçet's disease (BD) is a multisystemic disease of unknown etiology characterized by chronic relapsing oral–genital ulcers and uveitis. Multiple systemic associations including articular, gastrointestinal, cardiopulmonary, neurologic and vascular involvement are also observed [3, 4]. Although the etiopathogenesis of the disease remains unknown, increased neutrophil functions such as chemotaxis, phagocytosis and excessive production of reactive oxygen species (ROS), including superoxide anion, which may be responsible for oxidative tissue damage seen in BD, and also immunological alterations, and T lymphocyte abnormalities in both subpopulation and function have been considered to be correlated with the etiopathogenesis of BD [5, 6].

Enrolled in the study were 56 subjects with BD (35 women and 21 men), and 178 healthy control subjects (96 women and 82 men). The mean (±SD) age of the patients was 41.51 ± 12.41 years, and of the control subjects was 49.69 ± 14.01 years. All BD patients fulfilled the International Study Group criteria for the diagnosis of BD [7]. Control subjects were selected from among healthy persons without a history of malignancy, atopy, or autoimmune diseases. The study was approved by the Ethics Committee of Mersin University.

Blood was collected in EDTA-containing tubes and DNA was extracted from the lymphocytes using a high pure template preparation kit (Roche Diagnostics, Mannheim, Germany). The polymorphisms *GSTT1*, *GSTM1* and *GSTP1* were determined by real-time PCR with a LightCycler instrument using hybridization probes in combination with a LightCycler DNA Master Hybridization Probes kit (Roche Diagnostics). The PCR primers and the hybridization probes were synthesized by Tib Molbiol (Berlin, Germany). The sequences and the hybridization probes are shown in Table 1. The PCR conditions, essentially those described by Ko et al. [8], were as follows: 4 mmol/l $MgCl_2$, 0.2 μmol/l of each hybridization probe, 10 pmol of each PCR primer, 2 μl of the LightCycler DNA Master Hybridization Mix and 50 ng of genomic DNA in a final volume of 20 μl. These conditions were the same for the amplification of all three mutations.

The cycling program for GSTT1 comprised 45 cycles of denaturation (95°C, 0 s, ramp rate 20°C/s), annealing (66°C, 10 s, ramp rate 20°C/s) and extension (72°C, 19 s, ramp rate 20°C/s). After amplification a melting curve was generated by holding the reaction at 45°C for 10 s and than heating slowly (ramp rate 0.1°C/s) to 95°C. The programs for GSTM1 and GSTP1 were identical and comprised 45 cycles of denaturation (95°C, 0 s, ramp rate 20°C/s), annealing (66°C, 10 s, ramp rate 20°C/s) and extension (72°C, 7 s, ramp rate 20°C/s). After amplification a melting curve was generated by

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Table 1 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′-GTTGGGCTCAAATATACGGTGG-3′	5′-LCR640-ATGGCCGCTTCCCAGAAACTCTG-3′ 5′-TCACTCCTCCTTTACCTTGTTTCCTGCAAA-FL-3′
GSTT1	5′-TTCCTTACTGGTCCTCACATCTC-3′ 5′-TCCAGGTCAACCGGATCAT-3′	5′-LCR640-TCDAAGGCCGACCCAAGCTGGC-3′ 5′-CCGTGGGTGCTGGCTGCCAAGT-FL-3′
GSTP1	5′-ACCCCAGGGCTCTATGGGAA-3′ 5′-TGAGGGCACAAGAAGCCCCT-3′	5′LCR640-TGTGAGCATCTGCACCAAGGGTTGGGG-3′ 5′-TGCAAATACATCTCCCTCATCTACACAAC-FL-3′
β-Globin	5′-CAACTTCATCCACGTTCACC-3′ 5′-GAAGAGCCAAGGACAGGTAC-3′	

holding the reaction at 45°C for 15 s and than heating slowly (ramp rate 0.1°C/s) to 95°C. The fluorescence signal was plotted against temperature to give melting curves for each sample. Peaks were obtained at 59°C for the B allele and at 63°C for the A allele for GSTP1 genotype. The quantification program of LightCycler instrument evaluated the presence of either of GSTT1 and GSTM1.

Student's *t*-test was used to compare the ages of the two groups. The chi-squared test was used to compare two independent proportions. All values are presented as mean and standard deviation (SD). P values < 0.05 were considered statistically significant. The association between *GSTM1*, *GSTT1* and *GSTP1* polymorphisms, and BD was modeled through multivariate logistic regression analysis. The GST assays place individuals into distinct categories: those with present or null genotypes for *GSTM1* and *GSTT1*, and homozygous 105 Ile or heterozygous or homozygous 105 Val genotypes for *GSTP1*. Odds ratio and confidence intervals were used to analyze the frequency of occurrence of *GSTM1*, *GSTT1* and *GSTP1* genotypes in patients with BD compared to the control group. 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 104) functional alleles.

Patients with BD showed a higher prevalence of the GSTM1-null genotype than the healthy individuals (41.1% and 24.2%, respectively; OR 2.01, 95% CI 1.15–3.84). But the GSTT1(null) and GSTP1(val/val) gene polymorphisms were not associated with an increased risk of developing BD (OR 0.79, 95% CI 0.36–1.69; OR 0.49, 95% CI 0.10–2.31, respectively; Table 2). We analyzed the genotypes of GSTM1 and GSTT1 in combination to evaluate whether the combination of these genotypes is associated with the development of BD. No relationship was observed between the null combination of the GSTM1 and GSTT1 genotype polymorphisms and risk of BD (OR 1.85; 95% CI 0.52–6.59; Table 3).

In spite of an unknown etiology, it is now accepted that reactive oxygen species produced by neutrophils may be related to the pathogenesis of BD. Increased malondialdehyde and superoxide dismutase levels, but decreased glutathione peroxidase activities in erythrocytes have been observed in patients with BD [9, 10].

Table 2 GST genotypes and the risk of developing Behçet's disease

Variable	Cases ($n = 56$)	Controls ($n = 178$)	
	n (%)	*n* (%)	OR (95% CI)
GSTM1[a]			
Present	33 (58.9)	135 (75.8)	1 (referent)
Null	23 (41.1)	43 (24.2)	2.01 (1.15–3.84)
GSTT1[a]			
Present	45 (80.4)	132 (74.2)	1 (referent)
Null	11 (19.6)	46 (25.8)	0.79 (0.36–1.69)
GSTP1			
Ile/Ile	32 (57.1)	86 (48.3)	1 (referent)
Ile/Val	22 (39.3)	78 (43.8)	0.85 (0.45–1.62)
Val/Val	2 (3.6)	14 (7.9)	0.49 (0.10–2.31)

[a]Carriers of at least one intact allele are used as reference

Toxic compounds such as pesticides and insecticides have been incriminated in BD. Recently, Aynacioglu et al. have shown that the N-acetyltransferase 2*5B allele is slightly higher in patients with BD. This enzyme contributes to the metabolism of drugs and toxic compounds [11].

GST are a family of proteins that conjugate glutathione to various electrophilic substances and thus are thought to play an important role in the prevention of toxic injuries in various tissues. GST are present in the cytosol of most organs, whose expression is tissue specific. Liver, kidney, and muscle contain multiple forms of both the alpha and mu class of GST. The pi form isozyme is the predominant form in the placenta and is also found in the skin and in human keratinocytes [12]. In inflammatory diseases, the main source of ROS is inflammatory cellular infiltrate. Stimulated monocytes produce superoxide, the respiratory burst of infiltrating polymorphonuclear neutrophils in inflamed skin produces high local levels of superoxide anion and hydrogen peroxide. Production of nitric oxide by nitric oxide synthase also occurs in keratinocytes. Excessive pro-

Table 3 Distribution of GSTT1 and GSTM1 genotypes in combination

	Both null genotypes, *n* (%)	Others, n (%)
Patients ($n = 56$)	4 (7.1)	52 (92.9)
Control ($n = 176$)	7 (4.0)	169 (96.0)
OR (95% CI)	1.85 (0.52–6.59)	

duction of ROS results in peroxidation of cell membrane lipids and damage to proteins and DNA. The skin possesses considerable endogenous protection against oxidative damage, as it is equipped with several antioxidants. Reduced glutathione has gained attention as the central cellular antioxidant in the skin that also participates in the metabolism of xenobiotics and leukotriene synthesis [13].

In our study, patients with BD showed a higher prevalence of the GSTM1 null genotype than healthy individuals. Hereditary deficiencies of this enzyme activity may lead to an imbalance between pro- and antioxidant systems resulting in excessive ROS formation. ROS are important mediators of inflammatory and immune reactions in vivo, leading to oxidative cellular damage [14]. As shown in patients with BD, excessive ROS production by activated neutrophils and the presence of an impaired oxidant/antioxidant balance in both neutrophils and plasma may lead to oxidative damage by ROS, as reflected by decreased GST enzyme activity. Further studies on larger groups are needed to determine the prevalence of GST polymorphisms in patients with BD and to determine whether they constitute a major risk factor in the development of BD.

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