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Protective role of glutathione S-transferase P1 (GSTP1) Val105Val genotype in patients with bronchial asthma

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Background
Glutathione *S*-transferase P1 (GSTP1), the abundant isoform of glutathione *S*-transferases (GSTs) in lung epithelium, plays an important role in cellular protection against oxidative stress and toxic foreign chemicals. It has been suggested that polymorphisms in the GSTP1 gene are associated with asthma and related phenotypes. As significant interindividual and interethnic differences exist in the distribution of xenobiotic-metabolizing enzymes, we have studied the GSTP1 Ile105Val polymorphism in patients with asthma in a Turkish sample.

Methods
GSTP1 Ile105Val polymorphism in exon 5 was determined in 210 patients with asthma (112 extrinsic and 108 intrinsic) and 265 control individuals without lung diseases and without history of allergy or atopy, using polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) techniques.

Results
The proportion of GSTP1 Val105 homozygotes was significantly lower in the patients with asthma than in the control individuals (3.8% *vs* 12.1%). The odds ratio for GSTP1 Val105 homozygotes *vs* all other genotypes was 0.29 (95%CL 0.13–0.64, p = 0.01) for asthmatics. The distribution of GSTP1 Ile105Val genotypes and the frequency of GSTP1 Val105Val homozygotes (3.7% *vs* 3.9%) was not significantly different between extrinsic and intrinsic asthmatics.

Conclusion
These results suggest a significant association between GSTP1 Ile105Val polymorphism and susceptibility to asthma and that the GSTP1 Val105Val genotype may be protective against developing this disease.

Introduction

Asthma is a chronic disease characterised by reversible airflow obstruction and airway inflammation that affects many people all over the world with increasing morbidity and mortality, especially in developed countries [1]. The pathogenesis and aetiology of asthma is very complex and not fully understood, although an interaction of multiple genetic loci and a variety of environmental factors have been suggested as important determinants of this disease [2, 3].

Some of the many potential candidate genes that may be associated with asthma include receptor genes, such as the beta 2-adrenergic receptor [4], genes encoding proinflammatory cytokines (the interleukin-13 (IL-13) gene) as well as their receptors (IL-4R alpha) [5], genes involved in signal transduction, such as the human sig-

nal transducer and activator of transcription 6 (STAT6) [6, 7]. Furthermore, some studies have shown an association between asthma and polymorphisms of enzymes that play an important role in the biotransformation of exogenous and endogenous compounds, such as histamine *N*-methyltransferase [8] and *N*-acetyltransferase 2 [9, 10]. In addition, polymorphisms of glutathion S-transferase (GST) members have been suggested as individual susceptibility factors to lung diseases [11]. The predominant cytosolic GST expressed in the human lung, GSTP1, is a candidate gene, because of its role in cellular protection against oxidative stress. Recently, it has been shown that a valine (Val) to isoleucine (Ile) exchange at codon 105 (GSTP1 Val105/Val105) in exon 5 may protect against developing asthma [12–16]. Although the Val105 variant has higher catalytic efficiency for polycyclic aromatic hydrocarbon diol epoxides, its efficiency for 1-chloro-2,4-dinitrobenzene is lower compared to the Ile105 variant [17, 18].

This study was performed to find out whether the GSTP1 Ile105Val polymorphism had an impact on susceptibility to bronchial asthma, classified as in the intrinsic and extrinsic asthmatics of a Turkish sample.

Method

Subjects

The study population consisted of 210 consecutive bronchial asthma patients (108 diagnosed as extrinsic and 102 as intrinsic asthmatics; 156 female, 54 male; mean age 40.1 years; range 17–70 years), genotyped for *N*-acetyltransferase 2 (NAT2) acetylation status in a recent study [10], and 265 control individuals (184 female, 81 male: mean age 42.3 years; range 20–73 years) residing around Gaziantep, in South-East Anatolia, Turkey. There was no gender or age differences between the two groups ($P = 0.214$ and $p = 0.619$, respectively). Both groups were comparable in terms of ethnicity. Active smokers were excluded. All individuals gave written informed consent and the study was approved by the local ethics committee of the University of Gaziantep. The patients were unrelated atopic and nonatopic asthmatic outpatients of the Department of Pulmonology, Sahinbey Hastanesi, Gaziantep, Turkey. The diagnosis was based on medical history, physical examination, lung function tests and chest X-rays, skin 'prick' tests, and total immunglobulin E (IgE) level. The diagnostic criteria used to establish asthma definition was the protocol of The European Community Respiratory Health Survey (ECRHS) [19]. Patients showing at least one skin prick test positivity were defined as extrinsic asthmatics. Control individuals were selected from staff members of the Medical Faculty of Gaziantep and outpatients of other Departments of our Hospital without signs and symptoms of asthma and other lung diseases, and allergy or atopy on the basis of questionnaire responses.

Total IgE and prick test assays

Total IgE were determined by immulite® (Diagnostic Products Corporotion (DPC), C.A (USA) which is a chemiluminescent enzyme-labelled qualitative immunoassay technique (normal range 1.0–183 IU ml^{-1}). We have used Stallargenes-Pasteur allergen extracts including Dermatophagoides pteronyssinus, Alternaria, Cladosporium, cat epithelia, Olea europea, Parietaria officinalis and Phleum pratense for the prick test. The negative control contained phenolated glycerol-saline solution and the positive control histamine solution of 1 mg ml^{-1}. The results of the prick test were considered to be positive if the diameters of the indurations of both histamine and allergens were the same.

Identification of GSTP1 genotypes

DNA was extracted from leucocytes manually by standard 3-step phenol/chloroform extraction and stored at +4 °C until further analysis. *GSTP1* genotypes were determined by two previously described polymerase chain reaction restriction-fragment length polymorphism (PCR-RFLP) analyses [18, 20]. No direct sequencing was performed. Briefly, 176 and 329 base pair (bp) fragments containing the Ile105Val polymorphism site were digested by *Alw*26I (New England Biolabs, Schwalbach, Germany) for two hours at 37 °C and the RFLP products were separated by 3.5% agarose gel electrophoresis.

Statistics

The differences in *GSTP1* genotype and allele frequencies between patient and control groups were examined with the Chi-square and Fisher's two-sided exact test. The analysis were performed using SPSS program Version 10.1 (Chicago, Illinois, USA). *P*-values of <0.05 were considered to be statistically significant.

Results

Using the two PCR-RFLP methods for determining the GSTP1 Ile105Val polymorphism, all genotypes were identically determined without discrepant results. The frequency of GSTP1 Val105 homozygotes was found significantly lower in the group of patients with asthma than in the control individuals (3.8% *vs* 12.1%, $p = 0.01$) (Table 1). The odds ratio for GSTP1 Val105Val homozygotes *vs* all other genotypes was 0.29 (95%CL

Table 1
Frequencies of GSTP1 genotypes among asthmatics and control subjects and association of GSTP1 genotypes with asthma risk

	Asthma patients (n = 210)		Control subjects (n = 265)				
GSTP1 Genotypes	n	%	n	%	OR	95%CL	P
Ile105Ile	109	51.9	134	50.6	1	_	
Ile105Val	93	44.3	99	37.4	1.15	0.79–1.69	0.46
Val105Val	8	3.8	32	12.1	0.31	0.14–0.69	0.03
GSTP1 Alleles							
A (Ile)	311	74.0	367	69.2	1	_	
G (Val)	109	26.0	163	30.8	0.79	0.59–1.05	0.10

Table 2
Distribution of GSTP1 genotypes and alleles among patients with extrinsic and intrinsic asthma

	Extrinsic asthmatics (n = 108)		Intrinsic asthmatics (n = 102)				
GSTP1 Genotypes	n	%	n	%	OR	95%CL	P
Ile105Ile	55	50.9	54	53.0	1	_	
Ile105Val	49	45.4	44	43.1	1.09	0.63–1.89	> 0.05
Val105Val	4	3.7	4	3.9	0.94	0.23–3.87	> 0.05
GSTP1 Alleles							
A (Ile)	159	73.6	152	74.5	1	_	
G (Val)	57	26.4	52	25.5	0.95	0.62–1.48	> 0.05

0.13–0.64, $p = 0.01$) for asthmatics. In addition, the odds ratio for GSTP1 Val105 homozygotes *vs* Ile105 homozygotes was 0.31 (95%CL 0.14–0.69, $p = 0.03$) for asthma patients, whereas no statistically significant difference was detected between heterozygote asthmatics and control subjects. The distribution of GSTP1 Ile105Val genotypes and the frequency of GSTP1 Val105Val homozygotes (3.7% *vs* 3.9%) was not significantly different between extrinsic and intrinsic asthmatics (Table 2). However, the total number of asthmatics homozygous for the mutant allele was small. There was no statistically significant difference of GSTP1 allele distribution between asthma patients and control subjects as well as between extrinsic and intrinsic asthmatics.

Discussion

The production of reactive oxygen species (ROS) by several inflammatory cells, which participate in airway inflammation, may contribute to the epithelial damage of asthmatic airways [21]. Furthermore, genetic polymorphisms of xenobiotic-metabolizing enzymes leading to interindividual differences in the formation of protein adducts may result in a different susceptibility to chemically induced allergy and autoimmunity [22]. Thus, defects in detoxifying ROS may influence the development and severity of asthma. It has been proposed that GSTP1 is a candidate enzyme in protecting the epithelial cells against ROS and related toxic products [12, 13].

Indeed, polymorphisms of the GSTP1 gene have been associated with susceptibility to lung diseases, including chronic obstructive pulmonary disease (COPD) and asthma and related phenotypes [12–16, 23]. Recently, it has been found that the presence of the GSTP1 Val105Val genotype conferred a sixfold lower risk of asthma compared to the wild type GSTP1 Ile105Ile genotype and that the frequency of GSTP1 Val105Val genotype correlated negatively with severity of airway dysfunction [12].

In the present study, we have also found an association between the GSTP1 Ile105Val polymorphism and susceptibility to asthma in a Turkish sample consisting of 210 asthma patients classified as extrinsic and intrinsic asthmatics. As the prevalence of cigarette smoking is relatively high in Turkish subjects [24], we have

excluded active smokers to avoid potential confounding factors such as smoking habits. The frequency of GSTP1 Val105 homozygotes was significantly lower in patients with asthma than in control individuals (3.8% *vs* 12.1%, $p = 0.01$) and the odds ratio for GSTP1 Val105 homozygotes *vs* Ile105 homozygotes was 0.31 (95%CL 0.14–0.69, $p = 0.03$). In addition, the frequency of GSTP1 Val105Val homozygotes was not significantly different between extrinsic and intrinsic asthmatics (3.7% *vs* 3.9%), suggesting that the GSTP1 Val105Val genotype is protective not only of allergic asthma, but also of nonatopic, nonallergic asthma. However, it should be considered that, although these two major types of asthma could be distinguished according to disease onset, patient and family history, epidermal prick test, IgE levels, and allergen dependency, there is a wide overlap between extrinsic and intrinsic asthma. On the other hand, both allergic and nonallergic stimuli may lead to epithelial cell inflammatory response, which is an important biochemical feature of asthma [25]. Therefore, it seems to be possible that GSTP1 plays a role in allergic as well as nonallergic asthma subtypes by modulation of ROS production.

In conclusion, our results demonstrate a significant association between Ile105Val polymorphism in exon 5 of GSTP1 and susceptibility to asthma and that the GSTP1 Val105Val genotype might protect against developing this disease. Whilst the Ile105Val polymorphism may contribute but little to the asthma phenotype, it is possible that other polymorphisms such as the Ala114Val substitution in exon 6 might be relevant.

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