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ORIGINAL ARTICLE

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Glutathione-*S*-transferase μ and theta gene polymorphisms as new risk factors of atopic bronchial asthma

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Abstract The genetic polymorphism of glutathione-*S*-transferase M1 (GSTM1) and glutathione-*S*-transferase T1 (GSTT1) genes and the cytochrome P4501A1 gene responsible for xenobiotic conjugating enzymes of the phase II and phase I detoxification system were studied by PCR-RFLP in the blood spots of 109 patients with atopic bronchial asthma and 90 healthy individuals. GSTM1 gene deletion ($GSTM1^{0/0}$) was detected in 47.8% of individuals in the control group and in 76.1% of asthmatic patients. Individuals without the GSTM1 gene were at approximately 3.5–fold higher risk of developing asthma. The proportion of $GSTT1^{0/0}$ genotypes was significantly higher in the group of asthmatics (67.0%) than in controls (23.3%). The proportion of individuals with a deficiency in both GSTM1 and GSTT1 gene activity was more than four times higher in asthmatic patients than in the control group (54.1% and 12.2%, respectively). The frequency of the Ile-Val polymorphism of the CYP1A1 gene was similar in controls and asthmatic patients. This study shows the association of atopic bronchial asthma with $GSTM1^{0/0}$, $GSTT1^{0/0}$ genotypes.

Keywords Asthma · Glutathione-*S*-transferase M1 · Glutathione-*S*-transferase T1 · Cytochrome P4501A1 · Gene polymorphism

Abbreviations *CYP1A1*: Cytochrome P4501A1 · *GSTM1*: Glutathione-S-transferase M1 · *GSTT1*: Glutathione-S-transferase T1 · *PCR*: Polymerase chain reaction

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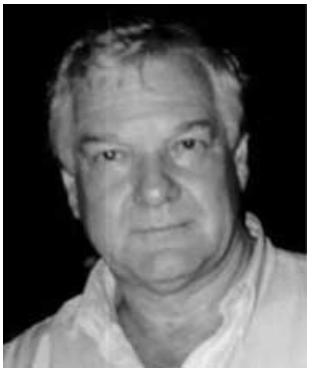

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Introduction

Allergic diseases affect approximately one-third of the general population. Asthma is a common heterogeneous disease, characterized by reversible airway obstruction and bronchial hyperresponsiveness and is commonly associated with atopy. The etiology and pathogenesis of asthma remain largely obscure. It is a complex multifactorial disease with an obvious genetic predisposition, immunological failure, and the possible involvement of noxious environmental factors. The search for genes in asthma has now led to several locations on the genome, including genes on chromosome 5, 11, and 12 [1, 2, 3]. The number of genes involved in this complex genetic disorders has not been fully determined.

Asthma occurs in 3.8 of 1000 individuals in Russia, and the figure rises to 5.2 in 1000 in St. Petersburg [4].

The high population prevalence of asthma led to investigations into the possible participation of detoxification system genes such as mEPHX and NAT2 in the pathogenesis of the illness [5, 6]. The polymorphism GSTP1 (enzyme of the phase II of detoxification) gene has been shown to be strongly associated with asthma, and this provides an alternative explanation for the linkage of chromosome 11q13 with atopy [7].

The enzymes of the detoxification pathway operate in several successive phases. Phase I is represented principally by the cytochrome 450 enzymes, which mediate oxidative metabolism, and by microsomal epoxide hydrolase and other enzymes involved in the detoxification pathway. Phase I chemical reactions may convert many harmless exogenous compounds into much more toxic or carcinogenic metabolites, which require immediate processing in phase II of the detoxification pathway. The phase II enzymes convert toxic intermediate electrophilic metabolites into polar, water-soluble, nontoxic derivatives which can then be excreted from the body. Thus phase I and phase II reactions should be well balanced.

Cytochrome P4501A1 (CYP1A1), which is expressed exclusively in human lung tissue is one of the phase I detoxification enzymes. CYP1A1 catalyzes bioactivation of environmental procarcinogens such as benzopyrene and polycyclic aromatic hydrocarbons [8].

GSTM1 and GSTT1 genes ensure synthesis of the enzymes that belong to the phase II detoxification enzyme system, responsible for biotransformation and degradation of certain electrophilic compounds such as *trans*-stilbene oxide, carcinogenic metabolites of benzopyrene (GSTM1) or dichlormethane, and ethylene oxide (GSTT1). Conspicuous genetic polymorphisms known for both of these genes are confined mainly to the presence of the nonfunctional null allele for GSTM1 and GSTT1 genes extending due to deletion resulting in no specific mRNA and protein products. The $GSTM1^{0/0}$ genotype is found in 40–50% of various populations [9], and the $GSTT1^{0/0}$ genotype occurs in only 10–30% of European populations [10]. All of these genes are known as risk factors predisposing to some environmentally induced diseases [11].

The principal goal of the present study was to analyze the distribution of GSTM1, GSTT1, and CYP1A1 deficient and active genotypes in patients with atopic bronchial asthma.

Materials and methods

Patients and controls

The study group comprised 109 patients of Russian origin and selected on the basis of atopic bronchial asthma who were attending the Institute of Pulmonology and St. Olga's Children's Hospital in St. Petersburg. All of the asthmatic subjects had asthma as diagnosed by specialists, with recurrent breathlessness and chest tightness, wheezing, and documented labile airflow obstruction, with variability in serial peak expiratory flow rates greater than 30%. In addition to standard spirometry and methacholine challenge testing, skin prick testing was performed to a standardized set of allergens which included mites, animal dangers, insects, and pollen. All asthmatics were atopic and sensitized to house dust. The group of asthmatic patients included 51 children younger than 15 years (47%) and 58 adults (53%). Age at onset was under 10 years in 52%, 11–20 years in 20%, 21–30 years in 15%, and over 30 years in 13% There were no heavy smokers among the subjects.

The control group consisted of 90 Russians living in St. Petersburg and northwestern Russia. Blood spots collected on the filter papers from each patient were used directly for polymerase chain reaction (PCR) with or without prior DNA extraction.

PCR and PCR-RFLP analysis

GSTM1 and GSTT1 gene polymorphism was analyzed as described elsewhere [12, 13] with slight modification. Coamplification of a 192-bp DNA fragment of the CYPA1 gene (A1R: GAACTGCCACTTCAGCTGTCT, A1F: GAAAGACCTCCCAGCGGTCA) was used throughout the study as a standard positive internal control for precise identification of $GSTM1^{0/0}$ and $GSTT1^{0/0}$ genotypes, which are identified as absence of the normal 271-bp DNA fragment typical for GSTM1 active genotypes ($GSTM1^{+/+}$ or $GSTM1^{+/0}$) or the 315-bp DNA fragment typical for GSTT1 active genotypes ($GSTT1^{+/+}$ or $GSTT1^{+/0}$).

A single PCR amplification was run with the primers (GSTM1 F GAA CTC CCT GAA AAG CTA AAG C; GSTM1 R GTT GGG CTC AAA TAT ACG GTG G and GST T1 F TTC CTT ACT GGT CCT CAC ATC TC; GST T1 R TCA CCG GAT CAT GGC CAG CA), 15 nM each in 25 µl amplification mixture plus 67 mM Tris HCl (pH 8.8), 16.6 mM ammonium sulfate, 6.7 mM MgCl2, 6.7 µM EDTA, 10 mM mercaptoethanol, 170 µg bovine serum albumin, 1.0 mM of each dNTP; 1 U *Taq* polymerase (Bion, Moscow). Denaturation (94°C, 7 min) was followed by 32 cycles of amplification: 94°C for 1 min, 53°C for 1 min, 72°C for 1 min and 20 s, and a final extension at 72°C for 7 min. The products were subjected to electrophoresis in 7.0% polyacrylamide agar gel, stained with ethidium bromide, and visualized under UV light (Fig. 1a, b).

CYP1A1 gene polymorphism was detected in one polymorphic site, A-G 462, as described [8], with some modifications for direct PCR analysis of the blood spots without DNA extraction. Amplification product was restricted with endonuclease *Hin*cII, and subjected to electrophoresis in 7.0% polyacrylamide agar gel, stained with ethidium bromide, and visualized under UV light. The undigested PCR product had a molecular length of 192 bp. The amplification product of homogeneous Ile type was cut into 144 and 48 bp, and Val/Val type into 125, 48, and 19 bp (Fig. 1c).

Statistical analysis

Genotype distribution and allele frequencies were compared between groups using the χ^2 test. The population attributable risk was estimated by standard methods. Statistical analyses were performed using Microsoft Excel (Microsoft Office 97) and the Statistica version 5.5a program.

Results

Table 1 shows the distribution of CYP1A1 genotypes in healthy controls and asthmatics living in northwestern Russia. The total number of the Ile/Ile genotype was 81 (90.1%) in the control group, that of the Ile/Val genotype was 9 (10.9%), and no one had the Val/Val genotype. The frequency of the Ile allele was 0.950 and that of the Val allele 0.050. Similar results were obtained in the asthmatic patients. We found 104 (95.4%) subjects with the Ile/Ile genotype, 5 (4.6%) with Ile/Val genotype

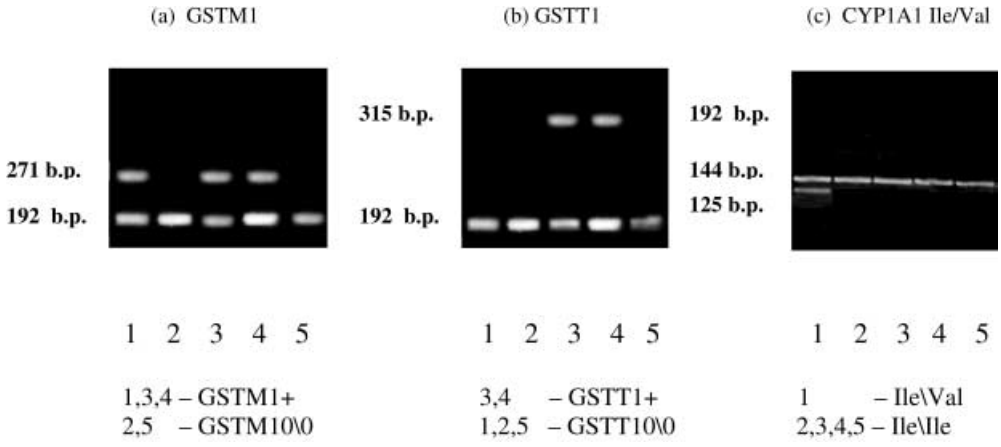

Fig. 1 Identification of the GSTM1, GSTT1 and CYP1A1 genotypes by PCR-RFLP analysis

Table 1 Frequency of normal and mutant CYP1A1 alleles in asthmatic patients and controls

	Genotypes			Allele frequencies	
	Ile/Ile	Ile/Val	Val/Val	Ile	Val
Patients (*n*=109)	104 (95.4%)	5 (4.6%)	0	0.977	0.023
Controls (*n*=90)	81 (90.1%)	9 (10.9%)	0	0.950	0.050

Table 2 Frequencies of the GSTM1 and GSTT1 0 homozygotes in asthmatic patients and controls

	$GSTM1^{0/0}$	$GSTT1^{0/0}$
Patients (*n*=109)	83 (76.1%)	73 (67.0%)
Controls (*n*=66)	43 (47.8%)	21 (23.3%)
χ^2	17.08, *P*<0.001	37.67, *P*<0.001

(χ^2=2.21, *P*=0.01373), and no one with the Val/Val genotype. The frequency of the Ile allele was 0.977 and that of the Val allele 0.023. There were no significant differences in the frequency of the Ile-Val polymorphism of CYP1A1 gene between controls and asthmatics.

The PCR methods used in our investigation allow to distinguish $GSTM1^{0/0}$ and $GSTT1^{0/0}$ subjects from relevant +/+ and +/0 subjects. We found 76.1% of asthmatic patients to have a GSTM1 deficiency (0 0) against 47.8% of controls (χ^2=17.08; *P*<0.001; Table 2). There was an effect associated with the GSTM1 null genotype (odds ratio 3.49, 95% Cl=1.93–6.37). The GSTT1 0 0 genotype frequencies were about three times higher in patients (67.0%) than in controls (23.3%; χ^2=37.67, *P*<0.001); the risk for GSTT1 null genotype was 6.66 (95% CI=3.64–12.21; Table 2).

The combined distribution of normal and mutant alleles of the GSTM1 and GSTT1 genes in the asthmatic patients and in the control group are shown in Table 3. According to the presence of active or mutant GSTM1 and GSTT1 alleles, all individuals examined were divided into four groups: (a) those with the $GSTM1^+$, $GSTT1^+$ genotype; (b) those with the $GSTM1^{0/0}$, $GSTT1^+$ genotype; (c) those with the $GSTM1^+$, $GSTT1^{0/0}$ genotype; and (d) those with the $GSTM1^{0/0}$, $GSTT1^{0/0}$ genotype. The "wild-type" genotype ($GSTM1^+$, $GSTT1^+$) was found in 41.1% of healthy donors but in only 11.0% of patients (χ^2=27.07, *P*<0.001). The proportion of asthmatic patients who were both $GSTM1^{0/0}$ and $GSTT1^{0/0}$ homozygous was 54.1% (59/109), as opposed to only 12.2% of controls (χ^2=37.97; *P*<0.001). The comparable odds ratio in the presence of both null genotypes was 8.47 (95% CI=3.6–19.9).

Discussion

Asthma, as many other multifactorial diseases, results from the interaction between adverse environmental factors and constitutional (genetic) resistance or susceptibility. The inflammatory process in the bronchi in atopic bronchial asthma stems from interaction of pulmonary epithelium with both blood cells and xenobiotics. This interaction provokes a high susceptibility and high reactivity of the bronchi – one of the basic symptoms of asthma. Thus asthma should be regarded as a multifactorial

Table 3 Summarized data on the distribution of normal and mutant GSTM1 and GSTT1 alleles in controls and in the asthmatic patients

	$GSTM1^+$, $GSTT1^+$	$GSTM1^{0/0}$, $GSTT1^+$	$GSTM1^+$, $GSTT1^{0/0}$	$GSTM1^{0/0}$, $GSTT1^{0/0}$
Patients (*n*=109)	12 (11.0%)	24 (22.0%)	14 (12.8%)	59 (54.1%)
Controls (*n*=66)	37 (41.1%)	32 (36.5%)	10 (11.1%)	11 (12.2%)
χ^2	27.07, *P*<0.001	4.47, *P*=0.03460	0.14, *P*>0.01	37.97, *P*<0.001

disease involving both a genetic predisposition and environmental factors.

We studied the Ile-Val polymorphism of the CYP1A1 gene in patients with atopic asthma and healthy individuals. The CYP1A1 normal and mutant allele frequencies in northwestern Russia corresponds to those in other European populations [14]. Our study has shown that the frequency of the Ile-Val polymorphism of CYP1A1 gene does not differ between asthmatic patients and controls.

GSTM1 and GSTT1 genes ensure synthesis of the enzymes that belong to the phase II detoxification enzyme system [15, 16]. The GSTM1$^{0/0}$/ and GSTT1$^{0/0}$/ alleles are distinguished in the phenotype as an absence of the relevant enzyme products, which are very important components of detoxification system. The homozygosity for null alleles of GSTM1 or GSTT1 genes is associated with increased susceptibility to environmental factors [17, 18]. The GSTM1$^{0/0}$, GSTT1$^{0/0}$ genotype frequency in healthy Russians (12.2%) is similar to that in the Spanish population frequency (12.5%) [19]. It should be noted that the two GST null genotype frequencies vary widely across ethnic groups: 8.8% in Egyptians, 6.3% in white Americans, 5% in Indians, 37% in Chinese, and 22% in Malays [19, 20, 21, 22].

We suggest that in the case of GSTT1 null alleles the absence of glutathione-*S*-transferase T1 enzyme plays a role in the bronchial asthma pathogenesis. The frequency of homozygotes for null alleles of both GST genes (GSTM1 and GSTT1) occurs in 54.1% of asthmatic patients and results in the loss of expression of these genes in the sick persons. Perhaps the loss of glutathione-*S*-transferase μ activity enhances the effect of θ enzyme absence.

Various hypotheses may be suggested to explain these findings. The enzymes of the detoxification pathway, which transform xenobiotics entering the body, operate in several successive phases of detoxification, and these phases should be well balanced [23]. Phase I of chemical reactions may convert many harmless exogenous compounds into much more toxic substances. As shown in our study, the distribution of normal and mutant alleles of the CYP1A1 gene, coding phase I enzyme, is the same in the asthmatic patients and in controls, but GST phase II enzymes are absent in 54% of the patients. It is possible that intermediate electrophilic metabolites, arising in the first phase of detoxification, are not utilized by GST enzymes in asthmatic patients and are not excreted. These intermediate metabolites may therefore attack cells and provoke the oxidative stress, influencing on the asthma pathogenesis.

Another function of glutathione-*S*-transferases concerns binding and transportation of steroid hormones [24, 26]. The advanced phase of allergic reaction in bronchial asthma patients (infiltration of eosinophils) is inhibited by corticosteroids, which are routinely used for the treatment of this disease [29]. Perhaps the absence of GSTT1 activity leads to impairments of the transportation of steroid hormones and results in increasing infiltration by eosinophils. Thus it is still very difficult to explain exactly the role of GSTs in a pathogenesis of atopic asthma. Our findings provide evidence for interaction between genetic and environmental factors involved in the pathogenesis of atopic asthma.

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