GENETICS

Relationship between Glutathione S-Transferase P1 Polymorphism and Bronchial Asthma and Atopic Dermatitis

O. G. Safronova, V. A. Vavilin, A. A. Lyapunova, S. I. Makarova, V. V. Lyakhovich, L. F. Kaznacheeva*, N. A. Manankin, O. A. Batychko*, and S. M. Gavalov***

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We determined the prevalence of *GSTP1-Ile*$_{105}$ and *GSTP1-Val*$_{105}$ alleles in patients with bronchial asthma and atopic dermatitis and healthy children of 2 groups (randomized and nonatopic control). The *GSTP1-Ile*$_{105}$/*Val*$_{105}$ genotype determines the resistance to atopic dermatitis (odds ratio=0.51; 95% confidence interval: 0.28-0.92; p=0.023). However, both homozygotes are at high risk of developing atopic dermatitis (near-significant differences).

Key Words: *bronchial asthma; atopic dermatitis; xenobiotics; glutathione S-transferase P1 polymorphism; predisposition*

Growing incidence of allergic diseases over the last 30-40 years [11] coinciding with environmental deterioration necessitates evaluation of the role of xenobiotic transformation in the pathogenesis and etiology of these disorders [1,3,6]. The superfamily of glutathione S-transferases (GST) holds much promise in this respect, since these enzymes play an important role in detoxification of electrophilic reactive metabolites of various xenobiotics and are involved in the metabolism of endogenous physiologically active substances. Published data show that genetic polymorphism affects activity of protein products [5]. These changes probably determine predisposition to various diseases.

Previously, we demonstrated the relationship between o-polymorphism in *GSTT1* and *M1* and bronchial asthma (BA) in children [2]. Here we studied the relationship between adenine/guanine substitution at position 313 of the primary *GSTP1* sequence leading to substitution of isoleucine 105 with valine ($Ile_{105}Val$) in the substrate-binding H-regions of the enzyme [8] and predisposition to BA and atopic dermatitis (AD).

MATERIALS AND METHODS

We examined 258 children patients Municipal Children's Emergency Hospital No. 1 (1 MCEH, 141 patients with atopic BA) and Municipal Children's Hospital No. 1 (1 MCH, 117 patients with AD). The control group included 96 nonallergic children (nonatopic control, Department of Traumatology, 1 MCEH) and 61 nonallergic children without clinical signs of allergy (randomized control, drug poisoning, 1 MCH). The patients were divided into age- and sex-matched groups. Observations were performed only with Europeans.

DNA was isolated from blood cells by the method of Kunkel. Ile_{105}/Val polymorphism of *GSTP1* was

Institute of Molecular Biology and Biophysics, Siberian Division of the Russian Academy of Medical Sciences; *Novosibirsk State Medical Academy; **Municipal Children's Hospital No. 1, Novosibirsk. ***Address for correspondence:*** drugsmet@.cyber.ma.nsc.ru. Safronova O. G.

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determined by polymerase chain reaction followed by restriction analysis [7]. A fragment of 176 base pairs was obtained after amplification and restricted with endonuclease BsoMA I (Sibenzim) at 55°C for 16 h. Fragments were separated in 7.5% polyacrylamide gel. Homozygotes Ile/Ile and Val/Val and heterozygote Ile/Val were characterized by 1 (176 b.p.), 2 (85 and 91 b.p.), and 3 bands, respectively.

The results were analyzed by means of EpiInfo 6 software. Genotype prevalence in groups were determined by χ^2 test with Yates correction. Two-tailed exact Fischer's test was used when needed. The probability ratio (odds ratio, OR) was estimated. Genotype frequencies relative to Hardy—Weinberg equilibrium (HWE) were determined using Hwe-Win software (M. B. Freidin, Institute of Medical Genetics).

RESULTS

GSTP1 polymorphism in Russian children (Europeans) (Table 1) of the randomized control group corresponded to the genotype distribution for control Europeans (database of the National Institute for Cancer Research, USA) [13]. Genotype distribution in randomized control children and patients with AD corresponded to HWE. In nonatopic control children and patients with BA both homozygotes negatively deviated from HWE ($p<0.05$). Pierson's test revealed no differences between genotype frequencies in patients and randomized control children. We evaluated statistical parameters for patients with BA ($\chi^2=2.49$, d.f.=2, $p=0.28$) and AD ($\chi^2=0.32$, d.f.=2, $p=0.85$). As differentiated from randomized control children, in nonatopic control group the polymorphism distribution did not correspond to HWE. Therefore, it was important to compare the data for these groups. However, the comparative study could not be performed by standard methods because of low incidence of *GSTP1Val/Val* genotypes in nonatopic control group. We assumed that the next two children of the nonatopic control group will have *Val/Val* genotypes, and the genotype frequencies were compared by Pierson's test. Despite a priori smoothening of the differences they were statistically significant ($\chi^2=6.04$, d.f.=2, $p=0.0489$).

It should be emphasized that the use of several control groups is a common method that facilitates the search for associations between candidate signs and diseases in the "case-control" studies [4]. In our study the comparison with parameters in randomized control group (better corresponding to populational characteristics by the HWE) revealed differences from the alternative state (no atopy), but not from atopic diseases. This increase in method sensitivity can be explained by high incidence of allergic diseases and subclinical manifestations of atopy in children [11]. Randomized control children could have subclinical manifestations of atopy. Therefore in further analysis we compared genotype distribution in groups of patients and nonatopic children. Statistical parameters for BA group were: $\chi^2=2.81$, d.f.=2, $p=0.245$ and for AD group: $\chi^2=7.232$, d.f.=2, $p=0.026$.

For identification of the genotype associated with high risk of atopic diseases OR were calculated (Table 2). We took into account the factor of passive smoking, which affects OR for genotypes *GSTM1* and *GSTT1* [2]. The heterozygous genotype was a statistically significant factor for the resistance to AD (OR=0.51; 95% CI: 0.28-0.92; $p=0.023$) and a near-significant factor for the resistance of passive smokers to BA. The homozygous *GSTP1Ile/Ile* genotype was a near-significant factor for high risk of AD in children not exposed to tobacco smoke (OR=2.76; 95% CI: 0.98-7.95; $p=0.057$). The homozygous *GSTP1Val/Val* genotype was a near-significant risk factor in the group of passive smokers (OR=3.73; 95% CI: 0.7-37.9; $p=0.165$). These features were not revealed for BA.

These results suggest that not all atopic diseases are associated with $Ile_{105}Val$ polymorphism in *GSTP1*. This is probably related to tissue-specificity of expressed GST. In the lungs not only GSTP1, but also GSTA1, GSTA2, GSTM1, and GSTM3 are expressed [9]. These forms of GST can mask the effects of GSTP1 due to substrate cross-specificity and higher catalytic activity for different substrates (by 10-50 times) [12]. In the skin GSTP1 is a predominant form of GST [10], and polymorphism of this gene can be of considerable importance. Moreover, BA is a complex clinical phenotype. Probably, it would be more appropriate to

TABLE 1. Frequency of Alleles and *GSTP1* Genotypes in Children

Group	Allele incidence		Genotypes (number of individuals, %)		
	Ile-allele	*Val*-allele	Ile/Ile	Ile/Val	Val/Val
Nonatopic control	0.651	0.349	32.0/33.3	61.0/63.5	3.0/3.1
Randomized control	0.689	0.311	29.0/47.5	26.0/42.6	6.0/9.8
BA	0.656	0.344	54.0/38.3	77.0/54.6	10.0/7.1
AD	0.671	0.329	51.0/43.6	55/47	11.0/9.4

TABLE 2. Relative Risk of Asthma and Dermatitis in Children with Various *GSTP1* Genotypes

Genotype, disease		Total	Nonsmokers	Passive smokers
GSTP1Ile/Val	BA	0.69 (0.39-1.22)	0.78 (0.28-2.15)	0.64 (0.32-1.30)
	AD	0.51 (0.28-0.92)	0.3 (0.10-0.85)	0.75 (0.35-1.60)
GSTP1Ile/Ile	BA	1.24 (0.70-2.22)	1.09 (0.38-3.10)	1.35 (0.66-2.70)
	AD	1.55 (0.85-2.81)	2.76 (0.98-7.95)	0.98 (0.45-2.10)
GSTP1Val/Val	BA	2.37 (0.59-13.70)	2.32 (0.2-118.1)	2.37 (0.4-24.7)
	AD	3.22 (0.81-18.40)	2.32 (0.2-128.4)	3.73 (0.7-37.9)

Note. Confidence intervals are shown in brackets.

study the relationship between GSTP1 polymorphism and quantitative characteristics of major manifestations (hyperreactivity, atopy, chronic inflammation, and neurogenic control). A. A. Fryer *et al*. [6] revealed a relationship between Ile alleles of GSTP1 and severe hyperreactivity of the bronchi in adult patients [6].

It should be emphasized that the ratio of heterozygotes in the nonatopic control group was higher than in the randomized control group (χ^2=5.79, p=0.016). These data indicate that GSTP1 plays an important role in the development of atopy. Homozygous genotypes are risk factors, but in nonsmokers this is wild-type homozygote, while in passive smokers this is mutant homozygote. The observed differences are probably associated with kinetic parameters of allozymes toward various substrates. Valine allozymes display higher catalytic activity toward diolepoxides of polycyclic aromatic carbohydrates, while isoleucine allozymes are more active toward 1-chloro-2,4-dinitrobenzene and alkylating agents [5].

The association of *GSTP1* polymorphism can be related to localization of the gene in region 11q13 linked to BA and its close proximity to another etiologically important gene located in this region (*e.g*., gene encoding the protein synthesizing by Clara cells in the lungs, CC16; or β-subunit genome of high-affinity receptors for IgE, FCER1B) [13]. However, we revealed a relationship with AD, but not with BA. Moreover, these associations were sensitive to tobacco smoke exposure, whose metabolism involves GSTP1. However, smoking had no effect on the relationship of FCER1B polymorphism with asthma and contents of total and specific IgE [14]. These data attest to independent role of GSTP1 in the formation of predisposition to atopic diseases.

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