Interaction of GSTM1, GSTT1, and GSTP1 Genotypes in Determination of Predisposition to Atopic Dermatitis

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 136, No. 10, pp. 438-441, October, 2003
Original article submitted May 7, 2003

The frequency of individual genotypes GSTM1, GSTT1, and GSTP1 and haplotypes was determined in patients with atopic dermatitis and healthy children. The actual frequency of some haplotypes was far below the theoretical value. Some haplotypes were associated with predisposition and resistance to atopic dermatitis.

Key Words: *glutathione S-transferases M1, T1, and P1; polymorphism; haplotypes; atopic dermatitis; predisposition*

Recent studies demonstrated the important role of xenobiotic biotransformation enzymes in the etiology of atopic diseases [1-3,5]. Various xenobiotics and endogenous bioactive compounds undergo consecutive transformations under the influence of phase 1 and 2 enzymes (cytochrome P450-dependent and conjugation reactions, respectively). Biotransformation system includes superfamilies of highly polymorphic genes for cytochrome P450, glutathione S-transferases (GST), sulfotransferases, and other compounds. Polymorphism and substrate cross-specificity determine high reliability of this system. However, genetic polymorphism results in the appearance of proteins with modified functional activity. Various combinations lead to xenobiotics detoxification/toxification imbalance and impair metabolism of endogenous substrates. Therefore, it is important to evaluate the effects of gene interactions on the resistance to pathogenic factors and predisposition to polyetiological diseases (*e.g.*, atopic dermatitis, AD). Here we studied the effect of interaction between polymorphism in *GSTM1*, *GSTT1*, and *GSTP1* genes associated with predisposition to atopic bronchial asthma [1-3,5].

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MATERIALS AND METHODS

Polymorphism of *GSTM1*, *GSTT1*, and *GSTP1* genes was determined in 325 children. AD was diagnosed in 126 patients (n=126) admitted at Municipal Children Hospital No. 1 (MCH No. 1). Control group included 99 nonallergic children (nonatopic control, Department of Traumatology, Municipal Children Emergency Hospital No. 1) and 100 nonallergic children without clinical signs of allergy (randomized control, drug-induced poisoning, MCH No. 1). The patients were divided into sex- and age-matched groups (1-15 years). Observations were performed only on Europeans.

DNA was isolated from blood cells by the method of Kunkel. Null-polymorphism of GSTM1 and GSTT1 was studied using polymerase chain reaction (PCR) as described elsewhere [7,8]. Ile_{105}/Val polymorphism of GSTP1 was evaluated by PCR [6] followed by restriction fragment length polymorphism analysis. Amplification product (176 b.p.) was restricted with endonuclease BsoMA I (Sibenzim) at 55°C for 16 h, and 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. Oligonucleotides were synthesized on a DNA ASM 800 device (Biosset). Genotype frequencies in groups were determined by χ^2 test with Yates correction. Two- and one-tailed Fisher exact tests were used when needed. Odds ratio (OR) was cal-

0007-4888/03/1364-0388$25.00

culated using EpiInfo 6 software. The maximum likelihood ratio was determined using SPSS 8.0 software.

RESULTS

The analysis of allele frequencies revealed increased frequency of null-allele GSTT1*0 in randomized control and, especially, in patients with AD (compared to nonatopic control group, Table 1). In randomized control and patients with AD the distribution of GSTP1 genotypes corresponded to Hardy—Weinberg equilibrium (HWE), while in nonatopic control we observed a negative deviation of both homozygotes and positive deviation of heterozygotes from HWE ($p<0.05$). Comparative analysis of genotype distribution revealed differences between nonatopic control and patients with AD (GSTT1, $\chi^2=7.08$, $p<0.01$), nonatopic and randomized control (GSTP1, $\chi^2=13.7$, $p=0.0011$), and nonatopic control and patients with AD ($\chi^2=9.22$, $p=0.0099$). Theoretical frequencies for haplotypes were calculated from the frequency of individual genotypes GSTM1, GSTT1, and GSTP1 in the randomized control group (Table 2). Significant differences were found in estimated and theoretical frequencies of some haplotypes. First, minimum frequencies were revealed for haplotypes M1"+"/T1"0/0"/P1 Val/Val ($p=0.0031$; 3.6-fold below the theoretical value $p=0.011$) and M1"0/0"/T1"0/0"/P1 Val/Val ($p=0.003$; 2.3-fold below the theoretical value $p=0.007$), which can be explained by reduced viability of carriers. Second, accumulation of mutant alleles in haplotypes was associated with their disappearance from nonatopic control, but these haplotypes were observed in patients with AD, which attests to their association with predisposition to AD (the differences in haplotype distribution were significant). Evaluation of linear trend of relative risk for combinations of genotypes including one, two, and three homozygote mutant genotypes relatively to combination of 3 wild-type genotypes (M1"+"/T1"+»/P1 Ile/Ile) confirmed our assumption ($\chi^2=3.67$, $p=0.055$). For combinations with one and two mutant genotypes OR were 1.25 (95% CI=0.49-3.15) and 7.48 (95% CI=0.9-340.5), respectively. The absence of ternary mutant haplotype in the control made impossible to determine OR. Its frequency in the sample was below the theoretical value and, therefore, the risk tended to infinity. The study of trends yielded averaged value of risk for several haplotypes, while the contribution of individual haplotypes could significantly vary. Calculation of the relative risk for individual GST revealed association between the predisposition to AD and GSTT1*0/0 genotype (OR=2.84; 95% CI=1.28-6.40). The heterozygous GSTP1 Ile/Val_{105} genotype was associated with the resistance to AD (OR=0.50; 95% CI=0.27-0.90). Compared to the heterozygous genotype, homozygotes GSTP1 Val_{105}/Val_{105} (OR=4.13; 95% CI=1.01-24.00) and GSTP1 Ile_{105}/Val_{105} (OR=2.01; 95% CI=1.10-3.66) were at high risk of developing the disease. These results are consistent with the negative deviation of both homo-

TABLE 1. Distribution of Alleles and Genotypes of GST in Children

Parameter	Control		AD
	nonatopic	randomized	
Allele frequency			
*GSTM1*0*	0.643	0.624	0.661
*GSTM1*1*	0.357	0.376	0.339
*GSTT1*0*	0.333	0.436^{++}	0.512^{+}
*GSTT1*1*	0.667	0.564	0.488
GSTP1-Ile_{105}	0.651	0.695	0.691
GSTP1-Val_{105}	0.349	0.305	0.309
Genotypes			
*GSTM1*0/0*	0.414 (41)	0.39 (39)	0.437 (55)
GSTM1+	0.586 (58)	0.61 (61)	0.563 (71)
*GSTT1*0/0*	0.111 (11)	0.19 (19)	0.262 (33)
GSTT1+	0.889 (88)	0.81 (81)	0.738 (93)
GSTP1 Ile_{105}/Ile_{105}	0.333 (33)	0.483 (50)	0.461 (59)
GSTP1 Ile_{105}/Val_{105}	0.637 (63)	0.424 (39)	0.453 (56)
GSTP1Val_{105}/Val_{105}	0.03 (3)	0.093 (11)	0.086 (11)

Note. Alleles: *p*, differences estimated by χ^2 test; $^{+}p<0.001$ and $^{++}p<0.05$ compared to the nonatopic control group. Genotypes: number of children is shown in brackets.

TABLE 2. Distribution of GST Haplotypes in Groups

Haplotype	Theoretical values	Estimated values		AD
		nonatopic control	randomized control	
M1"+"/T1"+"/P1 Ile/Ile	0.239	0.172 (17)	0.25 (25)	0.198 (25)
M1"+"/T1"+"/P1 Ile/Val	0.209	0.313 (31)	0.16 (16)	0.183 (23)
M1"+"/T1"+"/P1 Val/Val	0.046	0.03 (3)	0.07 (7)	0.049 (6)
M1"+"/T1"0/0"/P1 Ile/Ile	0.056	0.02 (2)	0.05 (5)	0.057 (7)
M1"+"/T1"0/0"/P1 Ile/Val	0.049	0.051 (5)	0.07 (7)	0.079 (10)
M1"+"/T1"0/0"/P1 Val/Val	0.011	0.0 (0)	0.01 (1)	0.0 (0)
M1"0/0"/T1"+"/P1 Ile/Ile	0.153	0.131 (13)	0.17 (17)	0.159 (20)
M1"0/0"/T1"+"/P1 Ile/Val	0.134	0.242 (24)	0.13 (13)	0.12 (15)
M1"0/0"/T1"+"/P1 Val/Val	0.029	0.0 (0)	0.03 (3)	0.032 (4)
M1"0/0"/T1"0/0"/P1 Ile/Ile	0.036	0.01 (1)	0.03 (3)	0.056 (7)
M1"0/0"/T1"0/0"/P1 Ile/Val	0.031	0.03 (3)	0.03 (3)	0.063 (8)
M1"0/0"/T1"0/0"/P1 Val/Val	0.007	0.0 (0)	0.0 (0)	0.008 (1)
Maximum likelihood ratio	18.037*	20.156^{+}	23.08^{++}	6.516^{o}

Note. *p=0.81 compared to theoretical frequency; ^{+}p=0.028 and ^{++}p=0.01 compared to the nonatopic control group; ^{o}p=0.837 compared to the randomized control group.

zygous GSTP1 genotypes in nonatopic control from HWE. It is probably related to kinetic differences between GSTP1-Ile and GSTP1-Val allozymes for various substrates. Valine allozyme displays higher catalytic activity in relation to diolepoxides of polycyclic aromatic carbohydrates, while isoleucine allozyme is more active for 1-chloro-2,4-dinitrobenzene and alkylating agents [4]. Therefore, the heterozygous genotype can be of maximum advantage. A comparative study of haplotypes M1"+"/T1"+"/P1 Ile/Val and M1"+"/T1"+"/P1 Ile/Val revealed a protective effect (OR=0.5, 95% CI=0.20-1.24). We estimated trend characteristics (χ^2=12.25, p=0.004) and determined association of haplotypes with the predisposition to AD (relative to the resistance factor, Table 3).

Association with the predisposition to AD was statistically significant for M1"0/0"/T1"0/0"/P1 Ile/Ile haplotype and high for M1"+»/T1"0/0"/P1 Ile/Ile, M1"0/0"/T1"0/0"/P1 Ile/Val, and M1"0/0"/T1"+»/P1 Val/Val haplotypes.

TABLE 3. Associations of Haplotypes with Predisposition to AD

Haplotype	Frequency in groups		OR (CI)
	AD	nonatopic control	
M1"+"/T1"+"/P1Ile/Val	25	17	1.0
M1"+"/T1"+"/P1Ile/Ile	23 (p=0.149)	31	1.98 (0.81-4.89)
M1"+"/T1"+"/P1Val/Val	6 (p=0.163)	3	2.7 (0.50-18.13)
M1"+"/T1"0/0"/P1Ile/Ile	7 (p=0.054)	2	4.72 (0.78-49.47)
M1"+"/T1"0/0"/P1Ile/Val	10 (p=0.163)	5	2.7 (0.71-11.33)
M1"+"/T1"0/0"/P1Val/Val	0 (p=0.68)	0	1.35 (0.02-109.16)
M1"0/0"/T1"+"/P1Ile/Ile	20 (p=0.159)	13	2.07 (0.79-5.52)
M1"0/0"/T1"+"/P1Ile/Val	15 (p=0.85)	24	0.84 (0.33-2.12)
M1"0/0"/T1"+"/P1Val/Val	4 (p=0.07)	0	6.74 (0.68-163.29)
M1"0/0"/T1"0/0"/P1Ile/Ile	7 (p=0.021)	1	9.43 (1.06-438.6)
M1"0/0"/T1"0/0"/P1Ile/Val	8 (p=0.067)	3	3.59 (0.74-22.89)
M1"0/0"/T1"0/0"/P1Val/Val	1 (p=0.41)	0	2.7 (0.17-80.32)

Note. p, differences estimated by one-tailed Fischer exact test.

Thus, the predisposition to AD increases with accumulation of homozygous mutant GST alleles in haplotypes. In our opinion the disappearance of low-frequency haplotypes with 2 and 3 mutant genes in children at the age >1 year is of considerable importance. The family of cytoplasmic GST includes 12 genes [4] whose products display cross-specificity for exogenous substrates. For example, cumene hydroperoxide is conjugated by all GST, 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene are conjugated by GSTα, GSTμ, and GSTπ [4]. That is why the fact that mutations in three genes led to various events up to elimination of carriers from the population is suprising. It should be emphasized that homozygous deletions in GSTM1 produce less significant effects compared to deletions in GSTT1 and mutations in GSTP1. PThis is probably due to the fact that GSTM, GST, and GSTP families include 5, 2, and 1 genes, respectively [4]. The data indicate that GST play an important role in metabolic processes and development of functional systems starting from embryogenesis.

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