Atopy Parameters in Asthmatic Children Increase with Accumulation of Null-Alleles of Glutathione-S-Transferase M1

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Atopy parameters (total IgE, skin prick test, and peripheral blood eosinophil count) in children with atopic bronchial asthma depend on the number of glutathione-S-transferase M1 mutant alleles in the genotype and on family history of asthma.

Key Words: *atopy; asthma; glutathione-S-transferase M1 polymorphism*

Atopy, the most significant component in the pathogenesis of bronchial asthma, is highly prevalent in the population (30-50%) [5,9]. Along with atopy, the contribution of many other genetic and environmental factors to the development of asthma has been persuasively demonstrated. Many studies demonstrated the role of xenobiotic biotransformation enzymes, *e.g.* glutathione-S-transferases (GST) in the formation of asthma predisposition [1,3,4,6,8]. The mechanisms underlying association of GST polymorphism and asthma are not studied. Presumably, they are linked with the involvement of these enzymes into the metabolism of xenobiotics and endogenous physiological compounds, inflammation transmitters, oxidative stress products [1,3,4,6]. Analysis of association of polymorphisms of xenobiotic biotransformation enzymes with asthma and its clinical features suggests that this association is based on the involvement of GST into the development and function of the immune system [1,6]. Classical markers of atopy directly pointing to reagin hyperproduction are positive skin tests (prick test; SPT), increased content of total and/or specific IgE, and increased count of peripheral blood eosinophils [10]. We studied the relationship of these quantitative parameters of atopy in children with the number of null alleles of *GSTM1* in the genotype and family history of atopic asthma (AA).

MATERIALS AND METHODS

A total of 100 children with AA observed at Allergology Room of Children's Health Center No. 1, Central Clinical Hospital, Siberian Division of Russian Academy of Sciences, and 268 family members of these children were examined. The presence of clinical picture of AA, total IgE >100 IU/ml, and pronounced sensitization to communal or pollen allergens were criteria for selection of children into the study group.

Examinations of patients included collection of allergological case history with evaluation of family history, clinical examination, measurements of total serum IgE, and SPT with communal, epidermal, pollen, and food allergens. SPT was carried out by the standard method. The diameter of the spot was measured in millimeters. Total IgE were measured by enzyme immunoassay using IgE-EIA-BEST-strip kits (Vektor-Best). Eosinophil count in the peripheral blood was evaluated routinely.

Modern concepts admit common pathogenetic me chanisms for all allergic diseases (AD) [7]. Therefore, if the parents had asthma, pollinosis, allergic rhinitis,

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TABLE 1. Distribution of *GSTM1* Genotypes in Children with AA (n=100)

Group	*GSTM1* genotype, number of patients			Incidence of alleles		χ^2	p
	$GSTM1^{0/0}$ (k=0)	$GSTM1^{0/+}$ (k=1)	$GSTM1^{+}$ ($M1^{0/+}$+$M1^{+/+}$)	null-allele	plus-allele		
1	15	2	5 (4+1) k=1.2	0.862	0.174	6.2^{+}	0.01^{+}
2	22	8	28 (20+8) k=1.3	0.616	0.384	4.72*	0.03*
3	5	3	12 (7+5) k=1.4	0.5	0.5	0.6^{o}	0.43^{o}

Note. For $GSTM1^{+}$ group the theoretically expected number of individuals with $GSTM1^{0/+}$ and $GSTM1^{+/+}$ is shown in parentheses; $^{+}$compared to group 3, *compared to group 1, ^{o}compared to group 2.

atopic dermatitis, or urticaria, the children were considered as having positive family history of AD. Depending on this, 3 groups of children with different parameters of hereditary burden (h) were distinguished: 1) 22 children, both parents suffered from AD (h=2); 2) 58 children one parent had AD (h=1); and 3) 20 children, parents had no AD (h=0).

DNA specimens for amplification were collected as described previously [11]. *GSTM1* polymorphism was evaluated by polymerase chain reaction [12]. This method distinguishes homozygotic deletion of *GSTM1* (null genotype, $GSTM1^{0/0}$) and plus-genotype ($GSTM1^{+}$) uniting the homozygotes and heterozygotes carrying *GSTM1* plus-allele. Familial analysis with consideration for the genotypes of first- and second-degree relatives differentiated homozygotes from heterozygotes in many cases. Hence, all children were distributed into 3 groups by the *GSTM1* genotype: $GSTM1^{0/0}$; $GSTM1^{0/+}$ heterozygotes; and mixed $GSTM1^{+}$ group uniting homozygotes by the plus-allele ($GSTM1^{+/+}$) and some undetected heterozygotes. In order to evaluate the relationship between atopy markers and the plus-allele dose in the patient's genotype, the mean number of plus-alleles per individual was calculated in the $GSTM1^{+}$ group. The incidence of *GSTM1* alleles was calculated according to the Hardy—Weinberg law, after which the expected numbers of heterozygotes and homozygotes were calculated with these values of incidence. The difference between the number of patients in the $GSTM1^{+}$ group and expected number of wild homozygotes represents the number of undetected heterozygotes. Their estimation helped to calculate the mean number of plus-alleles per individual in the mixed $GSTM1^{+}$ group (k).

TABLE 2. Quantitative Parameters of Sensitization in Children with AA with Consideration for *GSTM1* Genotypes in Groups with Different Family History of Allergic Diseases (M±m)

Parameter	*GSTM1* genotype	All children (n=100)	Group 1 (n=22)	Group 2 (n=58)	Group 3 (n=20)
Total IgE, IU/ml	All genotypes	532.45±204.90	694.3±176.0	537.2±165.9	340.5±180.7
	$GSTM1^{0/0}$ (k=0)	668.2±158.1***	767.67±97.80***	635.7±134.9***	453.0±100.6
	$GSTM1^{0/+}$ (k=1)	521.54±136.30**	622.5±137.9***	548.1±101.7*	383.33±155.00
	$GSTM1^{+}$ (k=1.2)	408.9±181.3	443.00±103.54	456.9±163.3	282.92±196.10
SPT, mm	All genotypes	7.81±2.84	10.00±2.73	7.74±2.61	5.60±1.67
	$GSTM1^{0/0}$ (k=0)	9.98±2.38***	11.20±1.97***	9.68±2.36***	7.60±1.52**
	$GSTM1^{0/+}$ (k=1)	7.54±1.80**	10.00±2.83***	7.75±1.67	5.33±0.58
	$GSTM1^{+}$ (k=1.3)	5.87±1.80	6.40±1.34	6.21±1.95	4.83±1.19
Peripheral blood eosinophils, %	All genotypes	7.42±3.37	9.36±3.47	7.76±2.90	4.3±2.4
	$GSTM1^{0/0}$ (k=0)	9.43±3.11***	10.67±3.20**	9.40±2.65***	5.8±2.2
	$GSTM1^{0/+}$ (k=1)	7.31±2.02*	8.00±1.41	8.25±1.28	4.33±0.58
	$GSTM1^{+}$ (k=1.4)	5.58±2.86	6.00±2.35	6.32±2.74	3.67±2.60

Note. *p<0.05, **p<0.01, ***p<0.001 compared to $GSTM1^{+}$.

TABLE 3. Regression Equations for Atopy Parameters Depending on the Number of *GSTM1* Plus-Alleles in the Genotype (*k*) in Groups with Different Family History

Group	Atopy parameter		
	total IgE	SPT	peripheral blood eosinophils (E)
1	IgE=789.78-262.50*k* *r*=-0.81**	SPT=11.25-3.43*k* *r*=-0.69**	E=10.69-3.64*k* *r*=-0.57**
2	IgE=655.38-146.20*k* *r*=-0.58**	SPT=9.84-2.69*k* *r*=-0.63**	E=9.88-2.57*k* *r*=-0.51**
3	IgE=480.55-121.30*k* *r*=-0.40	SPT=7.56-1.97*k* *r*=-0.72**	E=5.8-1.5*k* *r*=-0.38
Total	IgE=675.7-197.4*k* *r*=-0.60**	SPT=10.090-3.125*k* *r*=-0.68**	E=9.53-2.90*k* *r*=-0.59**

Note. *$p<0.05$, **$p<0.01$.

The results were compared using Student's *t* test.

RESULTS

Genotyping of 368 DNA specimens detected $GSTM1^{0/0}$ in 100 (27.2%) individuals, $GSTM1^{0/+}$ in 82 (22%), and $GSTM1^{+}$ in 187 (50.8%) individuals. Distribution of *GSTM1* genotypes in 100 children with AA, detected and theoretically expected heterozygote/homozygote ratio by plus-allele in $GSTM1^{+}$ subgroups were characterized by accumulation of $GSTM1^{0/0}$ with increasing *h* value, which was in line with known $GSTM1^{0/0}$ association with asthma [2,4] (Table 1). Sensitization characteristics in AA patients depended on the *GSTM1* genotype and *h* value. The increase in *k* was associated with a decrease, and increase in *h* with an increase in sensitization parameters (Table 2). This manifested in the whole sampling, being more demonstrative in groups 1 and 2. Quantitative description of these relationships was made using analyses of regressions and correlations (Table 3). The relationships of the studied characteristics of atopy with *k* and *h* were as follows: IgE=512.87-153.15*k*+128.00*h*; SPT=8.35-2.65*k*+1.36*h*; E=7.27-2.30*k*+1.78*h*.

Hence, the characteristics of atopy depend on both family history and number of $GSTM1^{0/0}$ in the genotype. The strength of the effects of these two factors is compatible, which is seen from close values of coefficients for *k* and *h* parameters. This fact indicates an important role of *GSTM1* and, presumably, the GST superfamily, in general, in the immune system functioning. It helps to explain the association of *GSTM1* polymorphism with predisposition to asthma observed in molecular epidemiological studies by its relationship with the key asthma phenotype — atopy.

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