Allele *NAT2*5* Determines Resistance to Bronchial Asthma in Children

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*NAT2*5* allele was found in 54.8% healthy children and 35.9% children with bronchial asthma, *i.e.* the risk of bronchial asthma was considerably lower in individuals carrying *NAT2*5* allele. *NAT2*5* allele manifests itself in both homo- and heterozygous states.

Key Words: *N-acetyltransferase; genetic polymorphism; bronchial asthma; predisposition*

Bronchial asthma (BA) is a complex multifactorial disease characterized by additive-polygenic inheritance, *i.e.* the disease manifests itself only when the influence of genetic and environmental factors reaches a threshold level [9]. High prevalence of BA and BA-associated mortality in the past 30 years are considered to be a consequence of impaired environmental conditions [1]. Therefore, it is important to study the pathogenetic mechanisms of BA and factors determining predisposition to this disease, which are activated by environmental conditions.

Arylamine N-acetyltransferase (N-AT, EC 2.3.1.5) plays an important role in the metabolism of endogenous (serotonin [10], dopamine [6], leukotriene E_4 [13]), and exogenous (acrylamines of exhaust gases, paints and varnishes, tobacco smoke [14]) compounds. Polymorphism of this enzyme is phenotypically manifested in the presence of fast and slow acetylators in human population. These two phenotypes differ in the risk of urine bladder and breast cancer, diabetes, and systemic lupus erythematosus [14]. Since acetylation reduces the toxicity of some xenobiotics and increased toxicity of others, N-AT-related risk varies depending on the chemical nature of pollutants. Hence, the N-AT genotype under different environmental conditions can represent either risk or resistance factor. Changes in N-AT activity are most often determined by single point mutations in the structural region of the *NAT2* gene. The most common mutation responsible for the slow-acetylator phenotype in Europeans is C_{481}-T substitution (allele *NAT2*5*) [12]. The prevalence of this mutation in different populations varies from 30 to 57% [15].

We focused on *NAT2* gene mutations as possible factors determining predisposition to BA, since N-AT is involved in metabolism of xenobiotics and endogenous compounds playing an important role in inflammatory processes and regulation of bronchial constriction. Moreover, it was shown that slow acetylators are characterized by an increased risk of allergic diseases, including BA [11]. However, the relationships between some alleles and the risk of BA have not yet been studied. N-AT allozymes exhibit different kinetic characteristics on different substrates [3], therefore, it seems important to clarify the role of *NAT2* allele in predisposition to BA.

MATERIALS AND METHODS

The study included 96 children with BA, 64 boys and 32 girls (66.7 and 33.3%, respectively) aging from 6.5 to 15 (mean age 10.8). They were divided into passive smokers (PS, n=56, those who lived with smoking parents or neighbors) and non-PS (NPS, n=40, those who were not exposed to tobacco smoke). The control group included 94 children, 55 boys and 39 girls (58.5

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and 41.5%, respectively) without signs of sensitization aged from 4 to 14 (mean age 8.2), also divided into PS (n=67, 71.3%) and NPS (n=27, 28.7%). All children were Europeoids, which excluded the effect of ethnic factors on the distribution of the examined polymorphic trait.

Amplification conditions for the assessment of *NAT2* gene polymorphism and oligonucleotide primers were similar to those described by A. Hubbard *et al.* [4]. PCR was performed in an AMP-105 amplificator. The obtained 547 bp fragment was treated with Kpn1 restrictase (Sibenzyme, Novosibirsk) followed by electrophoresis in 2% agarose. *NAT2*5* allele homozygotes yielded a single band corresponding to 547 bp. Homozygotes without this allele showed 2 bands corresponding to 433 and 114 bp, and heterozygote yielded all three bands.

The correlation between different *NAT2*5* genotypes and BA was evaluated by the risk ratio (RR) [9], showing the probability of becoming ill against the probability of remaining healthy for individuals with certain genotype. This parameter was calculated by the formula RR=(A/B)/(C/D), where A and C are the number of individuals with the given genotype among sick and healthy subjects, respectively, while B and D are the number of individuals without this genotype in the corresponding groups. RR calculated for the NPS subgroup characterizes the effect of genotype on the risk of BA. RR calculated for PS reflects the influence of smoking on the effect of the genotype. When calculating RR for sick PS and healthy NPS (RR_S), the effects of the genotype and smoking were added, so if they affect the risk in the same direction, the RR_S value is higher than in NPS, while if they act in different directions, it is lower. The data were processed using EpiInfo 6 software, the significance of differences was assessed with the χ^2 test.

RESULTS

Sick and healthy children differed in the incidence of *NAT2*5* allele. This allele was found in 54.8% healthy individuals, which agree with published data for European populations [15], while in the BA group only 35.9% individuals carried this allele. The distribution of *NAT2*5* genotypes in sick and healthy children and RR of BA for children with different genotypes are presented in Tables 1 and 2. Analysis of RR for the whole study group revealed that *NAT2*5* allele is a factor determining the resistance to BA, while the absence of this allele is associated with predisposition to this disease. The PS subgroup was characterized by lower RR compared to NPS, which means that under the influence of smoking BA develops even in individuals with resistant genotype. However, the difference between RR and RR_S was insignificant (Table 2).

The involvement of N-AT in the metabolism of xenobiotics is an important factor for the development of BA: smoking potentiates the negative effect of the absence of *NAT2*5*. This allele is known to encode an N-AT allozyme characterized by slow conversion of some substrates, in particular 2-aminofluorene [3]. The components of tobacco smoke 2-aminofluorene and heterocyclic amines become more aggressive upon acetylation [5]. It can be assumed that *NAT2*5* allele carriers less effectively metabolize these compounds, which determines less pronounced accumulation of toxic products and a lower risk of BA.

Predisposition to BA can be formed in another, presumably more important way associated with N-AT participation in the metabolism of endogenous substrates involved in sensitization, inflammation, and bronchial constriction. It has been reported that N-AT activity correlates with functional activity of immunocompetent cells [2]. N-AT participates in the metabolism of catecholamines and serotonin regulating bronchial constrictions, which is important in terms of predisposition to BA, since bronchospasm contributes to the mechanisms of bronchial obstruction [7]. Different N-AT allozymes are characterized by different affinity to a variety of substrates [5]. Low affinity to serotonin slows down its transformation into melatonin and accumulating serotonin can stimulate bronchospasm via D_2 receptors. Low affinity to dopamine can lead to the accumulation of dopamine, noradrenaline, and adrenaline stimulating bronchodilation via β_2-adrenoreceptors. Predominance of either processes depends on kinetic parameters of allozyme encoded by

TABLE 1. Distribution of *NAT2* Genotypes in BA Patients and Healthy Children

Genotype	Control			Patients with BA		
	PS (n=67)	NPS (n=27)	total	PS (n=56)	NPS (n=40)	total
Without *NAT2*5*	13	3	16	25	17	42
With *NAT2*5*	54	24	78	31	23	54
heterozygotes	38	14	53	21	18	39
homozygotes	16	10	25	10	5	15

TABLE 2. OR as a Function of NAT2*5 Allele Genotype

Genotypes	RR			RR_S
	PS	NPS	all	
Without NAT2*5	2.73*	5.91*	3.79*	6.45*
With NAT2*5	0.3*	0.17*	0.26*	0.15**
heterozygotes	0.43**	0.76	0.53**	0.56
homozygotes	0.75	0.24*	0.51	0.37

Note. Significant difference between patients and healthy subjects: *$p<0.01$; **$p<0.05$.

these alleles. We found no published data on the kinetic parameters of different allelic variants of N-AT with regard to serotonin and dopamine, but our findings suggest that *NAT2*5*-encoded allozyme possesses a lower affinity for dopamine compared to wild-type enzyme, while its affinity to serotonin is similar or higher.

Thus, the prevalence of *NAT2*5* allele in the control children population in Novosibirsk is the same as in other European populations and the presence of this allele is a factor of resistance to BA.

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