BIOPHYSICS AND BIOCHEMISTRY

Polymorphism of Arylamine-N-acetyltransferase 2 Gene Is Associated with the Risk of Atopic Dermatitis

S. I. Makarova, E. M. Dodunova, G. G. Ivanova, V. A. Vavilin, L. F. Kaznacheeva*,, and V. V. Lyakhovich**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 139, No. 6, pp. 628-631, June, 2005
Original article submitted December 8, 2004

A case—control study was conducted to evaluate the relationship between C481T and G590A polymorphisms of arylamine-N-acetyltransferase 2 and predisposition to atopic dermatitis in children. Double heterozygote 481C/T and 590G/A in girls is a factor of resistance to atopic dermatitis, especially in the absence of smoking-related effects.

Key Words: *atopy; atopic dermatitis; polymorphism; arylamine-N-acetyltransferase 2*

According to WHO reports, 10-28% people in economically developed countries suffer from allergic diseases. The contribution of atopic dermatitis (AD) to the structure of allergic diseases reaches 50-75%. It should be emphasized that 4.20-15.95% children have AD [8].

AD develops under the effect of environmental factors in the presence of genetic predisposition. A positive correlation exists between the degree of environmental pollution and incidence of AD [3]. The genes encoding xenobiotic-metabolizing enzymes are good candidates for determinants of genetic predisposition to AD.

Arylamine-N-acetyltransferase 2 (NAT2) catalyzes N-acetylation of aromatic and some heterocyclic amines probably acting as triggers of atopy development. Thirteenth point mutations were described in the primary sequence of this gene; their combinations form 29 alleles [6]. Allozymes for various substrates have different kinetic characteristics [7]. Little is known about the role of NAT2 in inflammatory and allergic reactions. A large discrepancy exists between published data on the relationship of NAT2 polymorphism with allergic diseases. Most previous studies concerned primarily bronchial asthma [2,3,9].

Here we studied possible relationship between predisposition to AD and existence of 2 common mutations in the NAT2 gene determining slow acetylation phenotype (C481T and G590A). Mutation C481T does not change amino acid sequence of the encoded protein. Mutation G590A leads to substitution of arginine-197 with glycine [6].

MATERIALS AND METHODS

We examined 87 children (1-15 years) with AD. They were hospitalized and observed at the Regional Allergic and Dermatological Center (Municipal Children's Hospital No. 1, Novosibirsk). The diagnosis of AD was verified by allergic anamnesis, clinical signs, and specific diagnostic methods (measurement of total IgG concentration, skin scarification test, and study of specific plasma IgG). The control group included 101 patients from the Traumatological Department that underwent control examination before discharge from the hospital. The absence of any sign of sensitization served as the main criterion for inclusion into the con-

Institute of Molecular Biology and Biophysics, Siberian Division of the Russian Academy of Medical Sciences; *Municipal Children's Hospital No. 1, Novosibirsk; **Novosibirsk State Medical Academy. ***Address for correspondence:*** makar@soramn.ru. S. I. Makarova

0007-4888/05/1396-0662

trol group. All patients and healthy children belonged to the Caucasoid race, which excluded possible effects of ethnic factors on polymorphous signs in the study groups. The children whose parent smoked at home were assigned to a group of passive smokers (PS). Other children entered the group of nonsmokers (NS).

NAT2 was genotyped in DNA samples from the peripheral blood. Polymerase chain reaction (PCR) was carried out using primers Nat7 (5' GGC TAT AAG AAC TCT AGG AAC-3') and Nat8 (5' AAT AGT AAG GGA TCC ATC ACC-3'). PCR was followed by hydrolysis with restriction endonucleases KpnI (identification of C481T substitution) and TaqI (study of G590A). Wild and mutant alleles were designated as 1 and 0, respectively. The mutant homozygote, wild-type homozygote, and heterozygote were designated as 00, 11, and 01 respectively. The study of genotypes with considering both mutations was performed with a 4-character code. The first pair of numerals designated genotype in position 481. The second pair of numerals designated genotype in position 590.

The distribution of NAT2 genotypes in groups was compared by Pearson test and χ^2 test. The relationship between genotypes and predisposition to AD was estimated by odd ratio (OR).

RESULTS

The incidence of C481T polymorphism in healthy children and patients was 0.546 and 0.529, respectively. The incidence of C590A polymorphism in healthy children and patients was 0.322 and 0.391, respectively. The estimated values are slightly higher compared to those in other Caucasian populations, including Russians from the Volga-Ural [1] and Voronezh [4] regions.

The incidence of double heterozygote for the studied mutations in patients was much lower than in healthy children (genotype 1010, OR=0.43, $p<0.05$, Fig. 1). Mutant homozygote was not revealed in healthy children (genotype 0000). This genotype probably determines high risk of developing AD, since it was found only in patients with AD (minimum OR, 6.16).

Genotype 1111 serves as a risk factor for AD in smokers (OR=3.9, Fig. 2). Carriers not exposed to the influence of smoking have equal probability of entering the group of patients or healthy children.

Analysis of distribution of NAT2 genotype in boys and girls revealed the presence of sex differences in patients, but not in healthy children (Table 1, Fig. 3). Genotype 1010 had a greater protective role in girls (OR=0.17, $p<0.05$). Moreover, differences were found in the frequency of genotypes 1111, 1100, and 1110.

These results indicate that the relationship between genotype and disease depends strongly on the

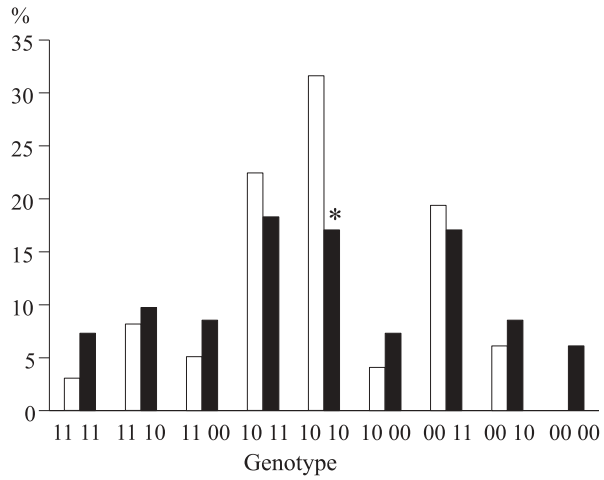

Fig. 1. Distribution of NAT2 genotypes in groups. Light bars: control; dark bars, atopic dermatitis. Here and in Fig. 3: *$p<0.05$ compared to the control.

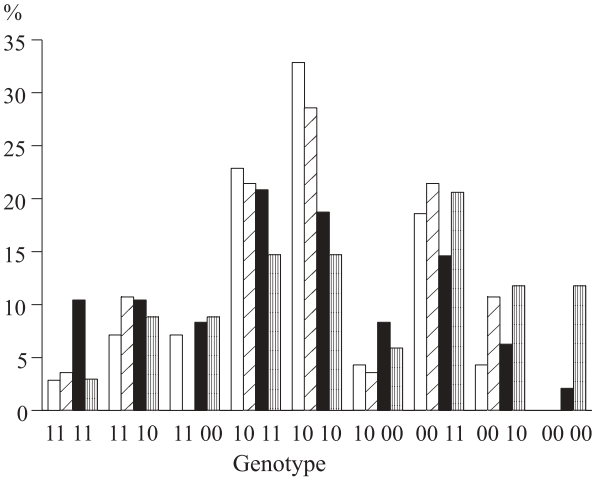

Fig. 2. Distribution of NAT2 genotypes in groups with different smoking status. Light bars: control, passive smokers. Slant shading: control, nonsmokers. Dark bars: atopic dermatitis, passive smokers. Vertical shading: atopic dermatitis, nonsmokers.

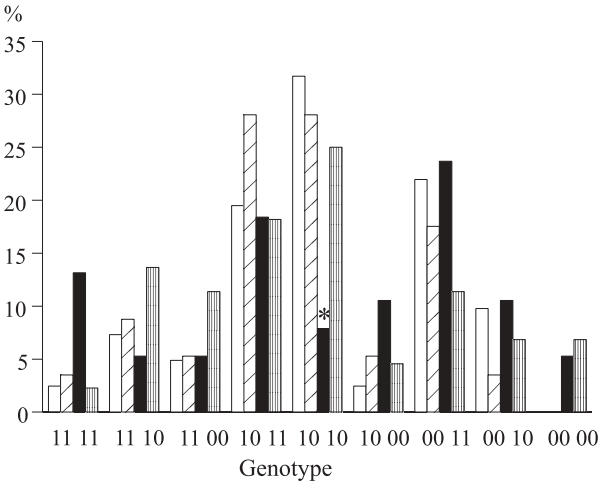

Fig. 3. Distribution of NAT2 genotypes in boys and girls. Light bars: girls, control. Slant shading: boys, control. Dark bars: girls, atopic dermatitis. Vertical shading: boys, atopic dermatitis.

TABLE 1. Distribution of NAT2 Genotypes in Groups

Group			Genotype according to KpnI restriction data								
			11			01			00		
			Genotype according to Taq1 restriction data								
			11	01	00	11	01	00	11	01	00
Girls	NS	control	0	2	0	1	6	0	2	2	0
		AD	1	1	0	0	1	2	4	4	2
	PS	control	1	1	2	6	8	1	7	2	0
		AD	4	1	2	7	2	2	5	0	0
Boys	NS	control	1	1	0	5	2	1	4	1	0
		AD	0	2	3	5	4	0	3	0	2
	PS	control	1	4	3	10	15	2	6	1	0
		AD	1	4	2	3	7	2	2	3	1

TABLE 2. Differences in the Distribution of NAT2 Genotypes in Groups

Accountable factors	Significance of differences	
	χ^2	p
Genotypes		
C481T	3.24	0.1978
G590A	4.61	0.0996
C481T and G590A	34.95	0.0005
Sex and genotypes		
C481T	7.33	0.3953
G590A	9.68	0.2076
C481T and G590A	51.12	0.0069
Smoking and genotypes		
C481T	12.74	0.0789
G590A	10.91	0.1425
C481T and G590A	53.07	0.0042
Sex, smoking, and genotypes		
C481T	23.69	0.1655
G590A	26.24	0.9454
C481T and G590A	98.29	0.0038

influence of exogenous factors (smoking) and patients sex. Our findings are consistent with published data that each polymorphous genetic sign is characteristic of the target group [5]. The genotype plays an important role in a certain group and determines high risk or resistance to the disease. Our study showed that NS-girls enter the target group for genotype 1010 (double heterozygote, OR=0.08, $p<0.05$). Contradictory data on the relationship between genetic polymorphism and disease can be explained by modifying effects of various factors on the effects of genes; moreover, target group cannot be identified before the study [3]. The significance of genetic differences between patients and healthy children increases, when we take into account even one of these factors (sex and smoking, Table 2). The study of genes for biotransformation enzymes, including NAT2, should consider the effects of smoking status, living in town or countryside, industrial factors, as well as sex and age, because gene expression varies with age and can depend on sex.

REFERENCES

1. T. V. Viktorova, G. F. Korytina, O. V. Makarova, *et al*., *Mol. Biol*., **37,** No. 6, 971-974 (2003).
2. V. V. Lyakhovich, V. A. Vavilin, S. I. Makarova, *et al*., *Vestn. Ros. Akad. Med. Nauk*, No. 12, 36-41 (2000).
3. H. Brocvielle, P. Muret, A. C. Goydadin, *et al*., *Skin Pharmacol. Appl. Skin Physiol*., **16**, No. 6, 386-392 (2003).
4. E. A. Gaikovitch, I. Cascorbi, P. M. Mrozikiewicz, *et al*., *Eur. J. Clin. Pharmacol*., **59**, No. 4, 303-312 (2003).
5. S. Garte, *Cancer Epidemiol. Biomarkers Prev*., **10**, No. 12, 1233-1237 (2001).
6. D. W. Hein, D. M. Grant, and E. Sim, *Pharmacogenetics*, **10**, No. 4, 291-292 (2000).
7. D. Hickman, J. R. Palamanda, J. D. Unadkat, and E. Sim., *Biochem. Pharmacol.*, **50**, No. 5, 697-703 (1995).
8. S. T. Holgate, *Nature*, **402**, No. 6760, Suppl. B, 2-4 (1999).
9. M. Nacak, A. S. Aynacioglu, A. Filiz, *et al*., *Br. J. Clin. Pharmacol*., **54**, No. 6, 671-674 (2002).