Analysis of Associations of *NAT2* Gene Polymorphisms with the Risk of Lung Cancer

M. V. Nikishina, V. A. Vavilin, S. I. Makarova, and V. V. Lyakhovich

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 143, No. 1, pp. 89-92, January, 2007
Original article submitted September 18, 2006

The incidence of 5 polymorphisms of N-acetyltransferase-2 gene was evaluated in patients with lung cancer. A803G polymorphism is a factor of lung cancer resistance in tobacco smoking Caucasians in Novosibirsk. Opposite effects of *NAT2* gene polymorphisms on the risk of lung cancer are possible.

Key Words: *NAT2; case-control study; genetic polymorphism; lung cancer; tobacco smoking*

Arylamine-N-acetyltransferase (EC 2.3.1.5) is an enzyme involved in biotransformation of xenobiotics, mainly aromatic and heterocyclic amines and hydrazines [4]. A total of 13 point mutations in the only encoding exon of *NAT2* gene (870 b.p.) were described [5]. Xenobiotic biotransformation enzymes play an important role in the formation of liability to polyfactorial diseases, including cancer. The percentage of slow acetylators in Caucasian populations is 40-70% [9], due to which the population risk of cancer is high even in the presence of low individual risk values. Associations of *NAT2* gene with the risk of some tumor diseases have been revealed [4], but the data on its association with lung cancer (LC), the most prevalent tumor disease, are contradictory [2].

We evaluated the incidence of 5 most prevalent polymorphisms of *NAT2* and analyzed associations of these polymorphisms with the risk of LC in Caucasians living in Novosibirsk.

MATERIALS AND METHODS

The study was carried out using DNA samples from the bank collected within the framework of the program "Health of Siberian Population". The group of LC patients consisted of 122 subjects (78 men and 44 women, mean age 60.4±10.4 years). Control group (no signs of tumor diseases) consisted of 167 subjects (119 men and 48 women, mean age 56.8±18.9 years). All examined subjects were Caucasians and were not relatives.

DNA was isolated from peripheral blood lymphocytes by phenol-chloroform extraction. PCR was carried out using Nat-Hu 14 and Nat-Hu 16 oligonucleotide primers [8] for obtaining a 1000 b.p. product. DNA amplification was carried out in 100 μl reaction mixture containing 60 mM Tris-HCl (pH 8.5 at 25°C), 1.5 mM $MgCl_2$, 25 mM KCl, 10 mM 2-mercaptoethanol, 0.1% Triton X-100, 50 μM each of deoxynucleoside triphosphates, 0.2 μM each of 2 primers, 20 U/ml Taq DNA-polymerase: 1 min at 94°C, 1 min at 56°C, and 1 min at 72°C (4 min first denaturation, 6 min final elongation).

TABLE 1. Incidence of *NAT2* Gene Polymorphisms

Polymorphism	Control	LC patients
C282T	0.323	0.340
G590A	0.329	0.307
C481T	0.410	0.402
A803G	0.410	0.348
G857A	0.027	0.025

Institute of Molecular Biology and Biophysics, Siberian Division of Russian Academy of Medical Sciences, Novosibirsk. ***Address for correspondence:*** mnikishina@rambler.ru. M. V. Nikishina

TABLE 2. Association of *NAT2* Gene Polymorphisms with the Risk of LC

Sign			Total group			Tobacco smokers			Nonsmokers		
			patients (*n*=22)	OR	control (*n*=167)	patients (*n*=61)	OR	control (*n*=88)	patients (*n*=61)	OR	control (*n*=79)
C282T	allele	C	161		226	78		128	83		98
		T	83	1.08 (0.76-1.53)	108	44	1.50 (0.92-2.47)	48	39	0.77 0.47-1.26)	60
	genotype	C/C	53	0.87 (0.55-1.40)	78	26	0.65 (0.34-1.25)	47	27	1.23 (0.62-2.42)	31
		C/T	55	1.14 (0.71-1.82)	70	26	1.18 (0.61-2.29)	34	29	1.08 (0.55-2.11)	36
		T/T	14	1.01 (0.49-2.10)	19	9	2.00 (0.70-5.71)	7	5	0.50 (0.17-1.50)	12
G590A	allele	G	169		224	85		125	84		99
		A	75	0.90 (0.63-1.29)	110	37	1.07 (0.64-1.77)	51	38	0.76 (0.46-1.25)	59
	genotype	G/G	59	1.24 (0.77-1.97)	72	30	1.06 (0.55-2.04)	42	29	1.48 (0.75-2.91)	30
		G/A	51	0.78 (0.49-1.25)	80	25	0.80 (0.41-1.54)	41	26	0.76 (0.39-1.49)	39
		A/A	12	1.11 (0.50-2.45)	15	6	1.81 (0.53-6.22)	5	6	0.75 (0.26-2.20)	10
C481T	allele	C	146		197	77		104	69		93
		T	98	0.97 (0.69-1.35)	137	45	0.84 (0.53-1.36)	72	53	1.12 (0.70-1.81)	65
	genotype	C/C	39	0.86 (0.52-1.41)	59	22	1.04 (0.52-2.05)	31	17	0.70 (0.34-1.45)	28
		C/T	68	1.40 (0.88-2.42)	79	33	1.29 (0.67-2.48)	42	35	1.53 (0.78-2.99)	37
		T/T	15	0.67 (0.34-1.31)	29	6	0.53 (0.19-1.46)	15	9	0.84 (0.33-2.13)	14
A803G	allele	A	159		197	88		106	71		91
		G	85	0.77 (0.54-1.08)	137	34	0.59* (0.36-0.96)	70	51	0.98 (0.60-1.57)	67
	genotype	A/A	53	1.56 (0.97-2.53)	55	32	2.24* (1.15-4.39)	29	21	1.07 (0.53-2.17)	26
		A/G	53	0.71 (0.44-1.13)	87	24	0.54 (0.28-1.05)	48	29	0.93 (0.48-1.81)	39
		G/G	16	0.86 (0.44-1.69)	25	5	0.63 (0.21-1.90)	11	11	1.02 (0.43-2.44)	14
G857A	allele	G	238		325	116		172	122		153
		A	6	0.91 (0.32-2.59)	9	6	2.22 (0.61-8.05)	4	0	—	5
	genotype	G/G	116	1.10 (0.38-3.18)	158	55	0.44 (0.12-1.62)	84	61	— —	74
		G/A	6	0.91 (0.31-2.62)	9	6	2.29 (0.62-8.49)	4	0	—	5

Note. *Significant difference in polymorphism incidence in the group ($p<0.05$).

Enzymatic hydrolysis of the amplified DNA fragment was carried out under optimal conditions for 3 h. The hydrolysis products were separated by electrophoresis in 2% agarose gel in Tris-acetate buffer. After electrophoresis DNA bands were detected by ethidium bromide staining and identified in UV by comparing them to the marker fragments. A set of restriction endonucleases (BamHI, Bst2UI, BstDEI, BstF51, TagI; SibEnzyme Firm) was used for detecting 5 most prevalent mutations: C282T and A803G (Lyz268Arg) [7], C481T [1], G590A (Arg197Gln), and G857A (Glu28-6Gly) [8].

Genotype incidences were verified for correspondence to the Hardy—Weinberg distribution using χ^2 test. The significance of differences in the incidence of signs in the groups was evaluated by the χ^2 test with Yates correction and Fisher exact test, if there were less than 5 cases in the reference group. The strength of associations of alleles and genotypes with LC was evaluated by odds ratio (OR) with a 95% confidence interval.

RESULTS

The distribution of genotype frequencies corresponded to Hardy—Weinberg equilibrium for all studied *NAT2* gene polymorphisms. A higher incidence of C282T and lower incidence of polymorphisms in positions 481, 590, 803, and 857 were observed in LC patients (Table 1). Analysis of associations of *NAT2* gene polymorphisms with LC risk showed that OR values for C282T, G590A, C481T, and G857A are close to 1 (Table 2). The OR=0.77 for A803G indicates a trend to a lower risk of LC (OR= 1.56 for "wild" homozygote). However, these differences are statistically insignificant.

The association between the sign and disease in the united groups can be weak and not reach the level of significance because of opposite effects of many neglected factors. Therefore we analyzed the associations with consideration for such an important risk factor as tobacco smoking. Significant differences in the incidence of A803G polymorphism in the subgroup of tobacco smokers (Table 2) were detected, indicating an association of A803G polymorphism with resistance to LC development under the effect of the tobacco smoking factor. The study of genotype distribution by this polymorphism showed a significant difference for "wild" homozygotes.

The OR values for C282T and G857 polymorphisms in the subgroup of tobacco smokers indicated a trend to predisposition to LC for carriers of these polymorphisms. Polymorphism C481T was associated with a trend to a lower risk, OR for this polymorphism homozygote was 0.53. The OR for G590A polymorphism is close to 1.

A trend to a lower risk was detected in tobacco smoking carriers of C282T, G590A, and G857A polymorphisms (OR=0.25 with admission of a solitary case in the heterozygote cell). The OR for A803G polymorphism was close to 1, slightly differing for C481T polymorphism (Table 2).

The impact of xenobiotic biotransformation enzymes in cancer is explained by their involvement in detoxication processes and activation of pro- and carcinogens entering the body. Polymorphism of genes encoding these enzymes can lead to imbalance of the xenobiotics detoxification/toxification processes because of changed activities of the enzymes. Changes in xenobiotic biotransformation caused by individual polymorphisms of the corresponding genes remain little studied, and therefore it is rarely possible to hypothesize the metabolic mechanisms explaining the observed associations between the polymorphism and predisposition to a certain disease. The kinetic parameters of acetylation reaction of several substrates for some allozymes were studied for *NAT2* [6], but even these results indicate that activity can change for the opposite one in the reactions with different substrates.

Significant associations with the risk of LC in tobacco smokers were detected in this study for A803G polymorphism. Despite the fact that this polymorphism leads to replacement of Lys268Arg in the protein amino acid sequence, the study of this protein in the yeast expression system showed that it did not differ from wild-type protein by stability, expression, and rate of acetylation of two test substrates (sulfamethazine and 2-aminofluorene) [3]. Changed functional activity of the allozyme towards other, heretofore not studied pro- and carcinogenic components of tobacco smoke can be hypothesized as the mechanism explaining the detected association. In addition, we should not rule out the hypothesis that A803G polymorphism is linked with another functionally significant sign.

Hence, our findings indicate that *NAT2* gene polymorphism A803G is associated with LC resistance in tobacco-smoking Caucasians in Novosibirsk. The OR values indicate possible opposite effects of *NAT2* gene polymorphisms on LC risk.

REFERENCES

1. M. V. Nikishina, A. G. Akishev, S. I. Makarova, *et al.*, *Biomed. Khim.*, **50**, No. 3, 269-272 (2004).
2. J. Borlak and S. M. Reamon-Buettner, *BMC Med. Genet.*, **7**, 58 (2006).
3. A. J. Fretland, M. A. Leff, M. A. Doll, and D. W. Hein, *Pharmacogenetics*, **11**, No. 3, 207-215 (2001).

4. D. W. Hein, *Toxicol. Lett.*, **112-113**, 349-356 (2000).
5. D. W. Hein, D. M. Grant, and E. Sim, *Pharmacogenetics*, **10**, No. 4, 291-292 (2000).
6. D. Hickman, J. R. Palamanda, J. D. Unadkat, and E. Sim, *Biochem. Pharmacol.*, **50**, No. 5, 697-703 (1995).
7. D. Hickman, A. Risch, J. P. Camilleri, and E. Sim, *Pharmacogenetics*, **2**, No. 5, 217-226 (1992).
8. D. Hickman and E. Sim, *Biochem. Pharmacol.*, **42**, No. 5, 1007-1014 (1991).
9. U. A. Meyer and U. M. Zanger, *Annu. Rev. Pharmacol Toxicol.*, **37**, 269-296 (1997).