Relationship between N-acetyl transferase-2 gene polymorphism and risk of bronchial asthma

Lülüfer TAMER[1], Mukadder ÇALIKOĞLU[2], Nurcan ARAS ATEŞ[3],
Hatice YILDIRIM[1], Sevim KARAKAŞ[3], Uğur ATİK[1]

[1] Mersin Üniversitesi Tıp Fakültesi, Biyokimya Anabilim Dalı,
[2] Mersin Üniversitesi Tıp Fakültesi, Göğüs Hastalıkları Anabilim Dalı,
[3] Mersin Üniversitesi Tıp Fakültesi, Tıbbi Biyoloji ve Genetik Anabilim Dalı, Mersin.

ÖZET

N-asetil transferaz-2 gen polimorfizmi ve bronşiyal astım riski arasındaki ilişki

*Allerjik hastalıkları da içeren birçok patolojik durumun mekanizması hala belirsizdir. Asetilasyon oranı, allerjik hastalıkların gelişmesini etkileyen bir faktör olabilir. Çalışmamızda NAT2 genetik polimorfizminin bronşiyal astımın gelişmesinde bir rolü olup olmadığını araştırmayı amaçladık. Çalışma grubumuz 97 bronşiyal astım hastası (atopik n= 62; nonatopik n= 35) ve 104 sağlıklı bireyden oluşmaktadır. Kan EDTA içeren tüplerde toplandı ve DNA "high pure template preparation" kiti ile lökositlerden elde edildi. NAT2*5A, NAT2*6A, NAT2*7A/B ve NAT2*14A allelleri LightCycler-NAT2 mutasyon belirleme kiti kullanılarak LightCycler cihazında gerçek zamanlı PCR ile saptandı. Genotipe göre, mutant NAT2*5A (OR= 3.84, %95 GA= 1.08-13.6) ve NAT2*6A (OR= 5.27, %95 GA= 1.06-26.05) genotipinin bronşiyal astımın gelişmesinde yüksek bir risk faktörü olabileceğini bulduk. Fenotiplere göre gruplandırıldığında; yavaş NAT2*5A asetilatör fenotipi hızlı fenotip ile karşılaştırıldığında bronşiyal astım oluşturma riski iki kat daha fazladır (OR= 2.7, %95 GA= 1.07-6.97). Bu çalışmamız NAT2 yavaş asetilatörün astım hastalığına karşı hassaslığın bir belirteci olabileceğini göstermektedir. Çalışmadan elde edilen bulgular, hastalığın patogenezini açıklamaya çalışan teoriler için olabileceği gibi terapötik amaçlar için de kullanılabilir.*

Anahtar Kelimeler: *Astım, NAT2, polimorfizm.*

Yazışma Adresi (Address for Correspondence):
Dr. Lülüfer TAMER, Mersin Üniversitesi Tıp Fakültesi, Biyokimya Anabilim Dalı,
33079, MERSİN - TURKEY
e-mail: lutamer@yahoo.com

SUMMARY

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Hatice YILDIRIM[1], Sevim KARAKAŞ[3], Uğur ATİK[1]

[1] Department of Biochemistry, Faculty of Medicine, Mersin University, Mersin, Turkey,
[2] Department of Chest Disease, Faculty of Medicine, Mersin University, Mersin, Turkey,
[3] Department of Medical Biology and Genetics, Faculty of Medicine, Mersin University, Mersin, Turkey.

*There are still uncertainties as to the mechanism of many pathological conditions, among them allergic diseases. It has been suggest that acetylation rate may be a factor that influences the development of allergic diseases. The aim of the present study was to investigate further whether the genetic polymorphism of the NAT2 plays a role in susceptibility to bronchial asthma disease. Ninety-seven patients with bronchial asthma (atopic n= 62; non-atopic n= 35) and 104 healthy individuals were participated in this study. DNA was extracted from the leucocyte by high pure template preparation kit. NAT2*5A, NAT2*6A, NAT2*7A/B and NAT2*14A polymorphisms of NAT2 were detected by using LightCycler-NAT2 mutation detection kit by real time PCR with LightCycler instrument. We found that mutant NAT2*5A (OR= 3.84, 95% CI= 1.08-13.6) and NAT2*6A (OR= 5.27, 95% CI= 1.06-26.05) genotype could be associated with a high risk for the development of bronchial asthma according to the genotype. After grouping phenotype, the risk for bronchial asthma was more than two times higher (OR= 2.7, 95% CI= 1.07-6.97) in individuals with the slow NAT2*5A acetylator phenotype compared to the fast phenotype. Our study suggests that the NAT2 slow acetylators may be a determinant in susceptibility to asthma disease. This finding may have implications for the theories for the pathogenesis of the disease as well as for therapeutic aspects.*

Key Words: *Asthma, NAT2, polymorphism.*

Interindividual and interethnic differences in the acetylation capacity of polymorphic arylamine N-acetyltransferase 2 (NAT2) affects therapeutic efficacy and the occurrence of side-effect of some clinically used drugs (1). Moreover, NAT2 is involved the metabolism of carcinogens from environmental, industrial and dietary sources (2). Polymorphic expression of arylamine N-acetyltransferase may be a differential risk factor in metabolic activation of arylamine carcinogenesis and susceptibility to cancers related to arylamine exposure (3). In addition, NAT2-acetylation polymorphism has been linked to susceptibility to certain autoimmune diseases such as systemic lupus erythematosus and scleroderma and allergic disease and atopy (4-7).

Allergic diseases affect approximately one-third of the general population. Asthma is a common heterogeneous disease, characterized by reversible airway obstruction and bronchial hyperresponsiveness (BHR) and is commonly associated with atopy. The etiology and pathogenesis of asthma remain largely obscure. It is a complex multifactorial disease with an obvious genetic predisposition, immunological failure and the possible involvement of noxious environmental factors (8).

It is possible that dietary, environmental factors and/or genetic polymorphisms in xenobiotic-metabolising enzymes may contribute to the development of the bronchial asthma disease. Therefore, the aim of the present study was to investigate further whether the genetic polymorphism of the NAT2 plays a role in susceptibility to bronchial asthma disease.

MATERIALS and METHODS

Study Subjects

The study population consisted of unrelated South Turkish white individuals. 104 healthy indivi-

duals who visited our hospital for an annual check up and hospital staff and 97 patients with bronchial asthma (atopic n= 62; non-atopic n= 35) were participated in this study. Control subjects were selected among healthy people with no history of cardiovascular disease, cancer, chronic degenerative neurological disease, chronic obstructive pulmonary disease, hepatitis, diabetes, hypertension, atopy, autoimmune diseases, allergies in general or alcohol abuse.

All the patients and the controls were undertaken to extensive history, physical examination, chest radiogram, complete blood count, routine biochemical investigation, skin prick test and pulmonary function tests. All the patients had both clinical history of asthma and positive reversibility test. Asthma was diagnosed according to the American Thoracic Society Statement (9). All the asthmatic subjects were clinically stable who had never experienced exacerbation of symptoms, away from systemic steroid usage and surgical treatment, trauma and had no signs suggestive of respiratory infection for at least 3 month before the study. Pulmonary function tests were performed by using Vmax 22 D (Sensormedix California, USA). Atopy was defined by the presence of a personal history of allergies, seasonal rhinitis, eczema, or allergic conjunctivitis and positive skin prick test responses skin reaction with a mean wheal diameter of > 3 mm larger than that produced with a saline control) with a panel of 13 common aeroallergens (Stallergenes SA, Pasteur, France). Demographics of the study population are given in Table 1. The study was approved by the Mersin University Ethics Committee on Human Research and each volunteer gave written informed consent.

Table 1. Characteristics of the study population.

	Patients n (%) 97 (100)	Controls n (%) 104 (100)
Age (years)	45.51 ± 11.84	49.10 ± 8.65
Sex		
Male	35 (34.7%)	57 (55.0%)
Female	62 (64.0%)	47 (45.0%)
FEV_1 (% predicted)	92.9 ± 19.8	104 ± 13.1
FVC (% predicted)	96.1 ± 13.5	101.1 ± 10.2
Atopy		
*Atopic	62 (63.9%)	0
Non-atopic	35 (36.1%)	104 (100%)

* Compared to non-atopic p< 0.05.

DNA Extraction and Genotyping of NAT2

Blood was collected in EDTA-containing tubes and DNA was extracted from the lymphocytes by high pure template preparation kit (Roche diagnostics, GmbH, Mannheim, Germany). NAT2*5A ($C^{481}T$), NAT2*6A ($G^{590}A$), NAT2*7A/B ($G^{857}A$) and NAT2*14A ($G^{191}A$) polymorphisms of NAT2 were detected by using LightCycler-NAT2 mutation detection kit by real time PCR with LightCycler instrument (Roche diagnostics, GmbH, Mannheim, Germany; catalog no: 3113914). The presence of mutations in both alleles of NAT2 was accepted as a slow acetylation phenotype. The wild types and heterozygous were termed as fast acetylators.

Statistical Analysis

Chi-square or Fisher's F exact tests were used to evaluate the distribution of the NAT2 genotypes among the asthma patients and control subjects. The association between NAT2 genotypes and asthma patients was estimated by computing odds ratios (OR) and 95% confidence intervals (CI) from logistic regression analyses. All statistical calculations were performed using the SPSS software package version (11.0 for Windows SPSS Inc., Chicago, IL). All tests were conducted at the p= 0.05 level of significance.

RESULTS

Characteristics of the study population are shown in Table 1. The distribution of each genotype for NAT2 in bronchial asthma cases and controls is shown in Table 2. The NAT2*5A mutant genotype were more frequent among asthma subjects with frequencies 16.5%, 2.9% for the cases and controls, respectively. On the other hand, NAT2*5A wild and heterozygous were observed with 30.9% and 52.6% frequencies, respectively, among asthma cases. Mutant NAT2*5A genotype could be associated

Table 2. NAT2 genotypes and the risk of developing asthma.

	Cases (n= 97)	Controls (n= 104)		
Variable	n (%)	n (%)	OR	95% CI
NAT2*5A				
Wild	30 (30.9)	41 (39.4)	1 (reference)	—
Heterozygous	51 (52.6)	56 (53.8)	1.61	0.59-4.36
Mutant	16 (16.5)	7 (6.7)	3.84	1.08-13.6
NAT2*6A				
Wild	58 (59.8)	60 (57.7)	1 (reference)	—
Heterozygous	30 (30.9)	40 (38.5)	1.78	0.69-4.61
Mutant	9 (9.3)	4 (3.8)	5.27	1.06-26.05
NAT2*7A/B				
Wild	65 (67.0)	60 (57.7)	1 (reference)	—
Heterozygous	26 (26.8)	41 (39.4)	0.58	0.32-1.07
Mutant	6 (6.2)	3 (2.9)	1.84	0.44-7.71
NAT2*14A				
Wild	54 (55.7)	57 (54.8)	1 (reference)	—
Heterozygous	41 (42.3)	46 (44.2)	1.62	0.69-3.77
Mutant	2 (2.1)	1 (1)	1.83	0.15-21.7

n: Number of sample, OR: Odds ratio, CI: (confidence interval) from conditional logistic regression. + Wild genotypes are used as reference.

with a high risk for the development of asthma (OR= 3.84, 95% CI= 1.08-13.6). The distribution of the NAT2*6A genotypes: wild, heterozygous, and mutant were 59.8%, 26.8%, 6.2% in the group with bronchial asthma and 57.7%, 38.5%, 3.8% in the healthy controls. In the cases group, the frequency of the NAT2*6A mutant genotype was higher in comparison with that of the control group and this increase was significant (OR= 5.27, 95% CI= 1.06-26.05). The distributions of genotypes of NAT2*14A and NAT2*7A/B were not statistically significantly different between the cases and controls.

When the asthma was categorized as atopic and non-atopic, patients with atopic asthma (56.5%) had a higher prevalence of the NAT2*5A heterozygous genotype than the non-atopic asthma patients (45.7%) (OR= 1.2, 95% CI= 0.31-4.50). Also we found 1.28, 0.8 and 1.2 fold increased risk of atopic asthma in individuals with NAT2*6A, NAT2*7A/B and NAT2*14A heterozygous genotype when compared with non-atopic asthma (OR= 1.28, 95% CI= 0.42-3.41; OR= 0.8, 95% CI= 0.21-3.11; OR= 1.2, 95% CI= 0.35-4.64), respectively but this increases were not significant. We was not found association between NAT2*5A, NAT2*6A, NAT2*7A/B and NAT2*14A mutant genotype and atopic asthma patients compared to the non-atopic asthma group (Table 3).

The frequencies of the slow and fast NAT2*5A acetylators were 16.5% vs. 83.5% and 6.7% vs. 93.3% in the patient and control groups, respectively (Table 4). The risk for bronchial asthma was more than two times higher (OR= 2.7, 95% CI= 1.07-6.97) in individuals with the slow NAT2*5A acetylator phenotype compared to the fast phenotype. There was no significant association between the NAT2*6A, NAT2*7A/B, NAT2*14A phenotypes and bronchial asthma.

When we investigated association between atopic and non-atopic asthma with NAT2 acetylator

Table 3. NAT2 genotypes and the risk of developing atopic asthma.

	Atopic (n= 62)	Non-atopic (n= 35)		
	n (%)	n (%)	OR	95% CI
NAT*5A				
Wild	20 (32.2)	10 (28.6)	1 (reference)	—
Heterozygous	35 (56.5)	16 (45.7)	1.20	0.31-4.50
Mutant	7 (11.3)	9 (25.7)	0.40	0.09-1.79
NAT*6A				
Wild	37 (59.7)	21 (60)	1 (reference)	—
Heterozygous	20 (32.3)	10 (28.6)	1.28	0.35-4.64
Mutant	5 (8.1)	4 (11.4)	1.18	0.17-7.90
NAT2*7A/B				
Wild	43 (69.4)	22 (62.9)	1 (reference)	—
Heterozygous	17 (27.4)	9 (25.7)	0.82	0.21-3.11
Mutant	2 (3.2)	4 (11.4)	0.27	0.03-1.98
NAT2*14A				
Wild	33 (53.2)	21 (60)	1 (reference)	—
Heterozygous	27 (43.6)	14 (40)	1.20	0.42-3.41
Mutant**	2 (3.2)	—	—	—

OR: Odds ratio, CI: (confidence interval) from conditional logistic regression.

* Carriers of at least one intact allele are used as reference.

** Odds ratio can not be calculated.

Table 4. The distribution of the mutations NAT2*5A, NAT2*6A, NAT2*7A/B and NAT2*14A as phenotypes in-groups.

Acetylator type	Patient group n (%)	Control group n (%)	OR 95% CI
NAT2*5A Fast	81 (83.5)	97 (93.3)	1 reference
Slow	16 (16.5)	7 (6.7)	2.7 1.07-6.97
NAT2*6A Fast	88 (90.7)	100 (96.2)	1 reference
Slow	9 (9.3)	4 (3.8)	2.5 0.76-8.59
NAT2*7A/B Fast	91 (93.8)	101 (97.1)	1 reference
Slow	6 (6.2)	3 (2.9)	2.2 0.53-9.13
NAT2*14A Fast	95 (97.9)	103 (99.0)	1 reference
Slow	2 (2.1)	1 (1.0)	2.1 0.19-24.3

n: Number of sample, OR: Odds ratio; CI: (confidence interval) from conditional logistic regression.

+ Fast acetylators are used as reference.

types, NAT2 fast acetylator with atopic asthma patients was higher frequencies than slow acetylator compared to non-atopic group but this increases were not significant (Table 5).

DISCUSSION

Several well-known drug metabolising enzymes catalyse the activation and detoxification of xenobiotics and are classified as phase I and II

Table 5. Association between atopic and non-atopic asthma with NAT2 acetylator types.

Acetylator type	Atopic (n= 62) n (%)	Non-atopic (n= 35) n (%)	OR 95% CI
NAT2*5A Fast	55 (88.7)	26 (74.3)	1 reference
Slow	7 (11.3)	9 (25.7)	0.3 0.12-1.09
NAT2*6A Fast	57 (91.9)	31 (88.6)	1 reference
Slow	5 (8.1)	4 (11.4)	0.68 0.17-2.71
NAT2*7A/B Fast	60 (69.4)	31 (62.9)	1 reference
Slow	2 (30.6)	4 (37.1)	0.25 0.04-1.48
NAT2*14A Fast	60 (96.8)	35 (100)	1 reference
Slow**	2 (3.2)	—	—

n: Number of sample, OR: Odds ratio, CI: (confidence interval) from conditional logistic regression.
+ Fast acetylators are used as reference.
** Odds ratio can not be calculated.

enzymes, respectively (10). Members of the phase I category include cytochrome P450-related enzymes and epoxide hydrolases. Phase II enzymes are N-acetyltransferases, glutathione S-transferases, UDP-glucoronosyltransferases and sulfotransferases (11).

Human arylamine N-acetyltransferases (NAT) are known to exist as two isoenzymes, NAT1 and NAT2, with different though overlapping substrate specificity (12). Interindividual differences in the generation of reactive metabolites, due to genetic polymorphisms of xenobiotic-metabolizing enzymes, including NAT2, may influence formation of protein adducts, which in turn may result in a different suspectibility to chemically induced allergy and autoimmunity (13).

Asthma, as many other multifactorial diseases, results from the interaction between adverse environmental factors and constitutional genetic) resistance or susceptibility. Inflammatory process in the bronchi in atopic bronchial asthma stems from interaction of pulmonary epithelium with both blood cells and xenobiotics. This interaction provokes a high susceptibility and high reactivity of the bronchi-one of the basic symptoms of asthma. Thus, asthma should be regarded as a multifactorial disease involving both a genetic predisposition and environmental factors (8).

Previous studies have indicated that the slow acetylation phenotype may be associated with allergic disease and atopy. Therefore the present study was performed to investigate further whether there is an association this polymorphism and bronchial asthma risk.

We found that mutant NAT2*5A genotype could be associated with a high risk for the development of bronchial asthma (OR= 3.84, 95% CI= 1.08-13.6) according to the genotype. In the cases group, the frequency of the NAT2*6A mutant genotype was higher in comparison with that of the control group and this increase was significant (OR= 5.27, 95% CI= 1.06-26.05). The distributions of genotypes of NAT2*14A and NAT2*7A/B were not statistically significantly different between the cases and controls. After grouping phenotype, the risk for bronchial asthma was more than two times higher (OR= 2.7, 95% CI= 1.07-6.97) in individuals with the slow NAT2*5A acetylator phenotype compared to the fast phenotype. There was no significant association between the NAT2*6A, NAT2*7A/B, NAT2*14A phenotypes and asthma. When we investigated association between atopic and non-atopic asthma with NAT2 genotype and acetylator types, there was no significant association between atopic asthma patients and non-atopic.

Vavilin et al. found that NAT2*5A slow acetylator risk for asthma whereas NAT2*6A remained unchanged in patients with bronchial asthma with passive smoker compared to healthy child-

ren (14). They concluded that xenobiotic-metabolizing enzymes are important development of bronchial asthma. Wikman et al. demonstrated that xenobiotic-metabolising enzymes including GST, NAT1 and NAT2 slow acetylators play role in inception of asthmatic reaction reactions related to occupational exposure to diisocynates (15). Similarly, Luszawska et al., Gawronska et al., Zielisska et al., Orzechowska-Juzwenko et al. and Nacak et al. show that NAT2 slow acetylator are increased for risk asthma (7,16-19).

Although arylamine N-acetyltransferases (NATs) are important in susceptibility to xenobiotic-induced disorders such as drug-induced autoimmune disease, allergy, atopy, cancer, but) their role in endogenous metabolism is yet to be elucidated (20).

It has been suggested that non-acetylated xenobiotics may accumulate in slow acetylators and subsequently metabolised by other enzymes into reactive intermediates. These reactive intermediates could alter self-proteins presented to the immune system and stimulate T cells which in turn initiate pathological and clinical signs of autoimmunity and allergic reaction.

In conclusion, our study with others suggests that the NAT2 slow acetylator status may be a determinant in susceptibility to asthma disease. This finding may have implications for the theories for the pathogenesis of the disease as well as for therapeutic aspects.

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