Lack of association of NAT2 (N-acetyl transferase 2) gene polymorphism with atopic asthma in Turkish subjects

Berrin Bagci Ceyhan, Ariadna Burgos*, Lyle J. Palmer, Jeffrey Drazen*
Department of Pulmonary Medicine, Marmara University School of Medicine, Istanbul, Turkey, *Department of Pulmonary and Critical Care Medicine and Harvard Medical School, Brigham and Women’s Hospital, Boston, Massachusetts, USA.

Introduction

There is compelling evidence that atopy is under substantial genetic control and that its expression depends on environmental factors. Asthma is a common and complex disease associated with atopy and is under the influence of multiple genetic and environmental factors. Although the current prevalence of self-reported asthma in the Turkish population was reported to be 7%, the genetics of asthma have not been studied in a general Turkish population.

NAT2 (N-acetyl transferase2) is one of most frequently phenotyped polymorphic metabolizing enzymes. NAT2 catalyzes the N-acetylation of various autocoids, therapeutic arylamine and hydrazine drugs, xenobiotics, and carcinogens from environmental industrial and dietary sources. The observation of hereditary polymorphisms resulting in slow and rapid acetylators focused interest on the role of these acetylators in modulating risk of malignancies, adverse drug effects, and other diseases. It is known that histamine inactivation is regulated in part by NAT2. Slow acetylators of NAT2 have been affected more often than fast acetylators by side effects of NAT2 substrates, possibly due to toxic metabolites formed in an alternate pathway. Previous studies in Poland have reported that slow acetylation is a risk factor for allergy. First, Patkowski et al. studied acetylation phenotype and found slow acetylation in 80% of subjects with chronic allergic rhinitis. Zielinska et al. found slow acetylation in 91% of allergic patients, and Gawronska-Szklarz et al. reported that 85% of allergic patients had slow acetylation. However, since NAT2 polymorphisms have not been broadly investigated in allergic asthma in...
Lack of association of NAT2

different ethnic groups, and its influence on clinical severity still remains unknown.

Our aim was to investigate the association of the NAT2 phenotype inferred from N-acetyl polymorphism with asthma in a case-control study of Turkish adults and to assess the relationship between allelic variation in the NAT2 gene and the severity of the asthma.

Materials and Methods

Patients

Fifty seven (45 female, 12 male) unrelated atopic asthmatic subjects and 56 (44 female, 12 male) unrelated healthy subjects were included in this study. All patients gave written informed consent to take part in the study. The study protocol was approved by Marmara University Hospital Ethics Committee. Controls were recruited from students and workers in the university who had no atopic or pulmonary symptoms. Among the controls, an allergic diathesis was excluded by history, physical examination, normal lung function test, negative skin-prick test, and normal IgE level. The atopic asthma phenotypes were characterized on the basis of medical history, physical examination, skin tests to common aeroallergens, total IgE levels, pulmonary function tests, and—unless baseline FEV1 was < 70 predicted—a methacholine challenge test. To qualify for the study, patients had to fulfill following criteria: a positive skin test to common aeroallergens and/or total serum IgE level of >100 IU/ml and a diagnosis of asthma based on asthma guidelines.14 The lung-function measurements (baseline and methacholine challenge test) were performed with standard equipment (Sensormedics, S3513, CA, USA) and techniques.15

Skin reactivity to common allergens (grass and tree pollens, mold, house dust mite, and animal dander (Center Laboratories, Port Washington, NY, USA) was measured by standard techniques in all subjects.16 The skin tests were considered to be positive if the diameter of the wheal was ≥3 mm larger than that of a negative control. Both negative (the solution used to prepare the allergen extract) and positive controls (histamine solution of 1 mg/ml) were used.

Total serum IgE levels were measured by ELISA (Pharmacia, Sweden). Patients were diagnosed as having mild or severe asthma according to published NIH asthma guidelines using frequency of exacerbations and symptoms, nocturnal symptoms, degree of exercise tolerance, and FEV1 values.14

DNA extraction

DNA was extracted from blood samples according to standard extraction protocols (QIAamp Blood Kit; Qiagen, Chatsworth, CA).

Identification of Mutations

Polymerase chain reaction (PCR). The primers used for the detection of NAT2 were forward: 5'AGGAAATCAAATGCTAAAGTATGATA-3' and reverse: 5'CTAGCATGAATCACTGCTTTC-3'

The PCR mixture (25 ml) contained 200 ng of genomic DNA, 10 pmol of each primer, 2.5 ml of 10X PCR buffer (Boehringer Mannheim, Mannheim, Germany), 2.5 ml of a 2 mM solution of deoxynucleoside triphosphates (adenosine, cytidine, thymidine, and guanosine), and 1.5 units of Taq polymerase. The PCR conditions were as follows: samples were heated to 94° C for 6 minutes and then underwent 35 cycles of 94° C for 1 minute, 59° C for 1 minute and 72° C for 1 minute, followed by 72° C for 6 minutes in a PTC-100 (MJ Research, MA, USA). A 15-ml aliquot of PCR product was electrophoresed on a 1.5% agarose gel, with the product visualized using ethidium bromide. A 1198-bp fragment was amplified and then digested with restriction enzymes.

Restriction fragment length polymorphisms (RFLPs). TspRI (New England Biolabs, Beverly, MA) was used to identify the mutation in position 341 of the NAT2 gene. The digestion mixture was added to 15 ml of the PCR product for a final volume of 30 ml. The digestion mixture included 10 U of TspRI, 3.0 ml of 10X NEBuffer 4, and 0.3 ml of 100X bovine serum albumin (100 mg/ml). Digestion with restriction enzyme was allowed to proceed for a total of 16 hours at 65° C. The fragments measuring 467, 374, and 357 bp for the T allele and 438, 374, 357 and 29 bp for the C allele were separated in 1.5% agarose gel and visualized with ethidium bromide. FokI (New England Biolabs) was used to identify the mutation in position 282 of the NAT2 gene. The digestion
mixture was added to 15 ml of the PCR product for a final volume of 25 ml. The digestion mixture included 4 U of FokI and 2.5 ml of 10X NEBuffer 4. Digestion with restriction enzyme was allowed to proceed for a total of 3 hours at 37° C. The fragments measuring 758, 288, 122, and 30 bp for the T allele and 429, 329, 288, 122, and 30 bp for the C allele were separated in 2.5% agarose and visualized with ethidium bromide. Genotyping results were confirmed by sequencing in 3 samples.

Statistical Analysis

The primary dichotomous outcome variable of the association analyses was case-control status for allergic asthma. Secondary analyses within the case group examined asthma severity (0=mild/moderate; 1=severe) and duration of disease as outcomes. The principal explanatory variables were dichotomous NAT2 acetylation phenotype (slow=1, rapid=0). Sex (1=male, 2=female), treatment for asthma (0=none, 1=beta-agonists only, 2=beta-agonists plus antiinflammatory drugs), and age were included as covariates in the multivariate models.

Generalized linear models (logistic and linear regression) were used to model the effects of multiple covariates and NAT2 acetylation phenotype on case-control status, asthma severity, and duration of disease. Both forward and backward stepwise modeling procedures were used to select a useful subset of independent predictors of case-control status. Checks of goodness of fit included an investigation of the need for interaction or polynomial terms, analyses of Pearson residuals, and examination of the effect of observations with high regression leverage.

Comparisons of sex, age, IgE concentrations, eosinophilia, and lung function indices were analyzed by t-test and we performed chi-square tests to compare counts by using Minitab v13 (Minitab) and S-Plus 2000 (Mathsoft). Statistical significance was defined at the standard 5% level.

Results

All asthmatic patients had positive skin-test results and were nonsmokers. The characteristics of the cases and controls are given in Table 1. Case subjects had significantly higher total serum IgE levels and eosinophil counts and significantly lower percent predicted FEV1 levels than controls (Table 1). Twenty three (40%) of the patients were defined as having severe asthma. We found that the mean PC20 value was 4.55 ± 5.37 mg/ml in subjects with mild asthma The mean age and the sex ratio were not significantly different between cases and controls. A family history of asthma was significantly more common in case subjects (Table 1).

Bivariate analysis suggested that case-control status was unrelated to the presence of the NAT2 slow acetylation phenotype ($C^2=0.08$, $P=0.77$). Multivariate analysis confirmed this finding (Table 2); independently of the other possible covariates, there was no evidence of a significant association between NAT2 acetylation phenotype and allergic asthma. The frequency of homozygous fast, heterozygous fast, and homozygous

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<tr>
<th>Table 1: Characteristics of study population</th>
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<tr>
<td>Number</td>
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<td>Sex (M/F)</td>
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<td>Mean age (yrs.)</td>
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<td>Mean FEV1 (percent predicted)</td>
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<td>Serum IgE level (IU/mL)</td>
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<td>Peripheral eosinophilia (%)</td>
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<td>Family history of asthma in primary relatives (%)</td>
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Mean±/SD; NS: Nonsignificant

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<th>Table 2: Association of NAT2 slow acetylation phenotype with asthma, asthma severity and duration of disease. Results from multivariate generalized linear modelling</th>
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<td>Outcome</td>
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<tr>
<td>Asthma*</td>
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<td>Asthma severity*</td>
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<td>Duration of disease (years)*</td>
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*Asthma case-control status. Model also adjusted for age, sex and education status. *Analysis within case group only. Model also adjusted for sex and age. cRegression coefficient [b] (SE)
slow acetylators was 11%, 42%, and 47% in the asthmatic group and 5%, 52%, and 43% in the control group, respectively.

Within the case population, bivariate analyses did not suggest any significant association between NAT2 acetylation phenotype and either asthma severity (c² = 0.08, P = 0.77) or duration of disease (mean_cases = 9.7 [SE = 1.8]; mean controls = 15.6 [SE = 2.9]; t = 1.73, P = 0.09). Multivariate analyses confirmed these results (Table 2).

Discussion

In this case-control investigation of Turkish patients with allergic asthma and control subjects, we assessed the association of allergic asthma with the genetic polymorphism of NAT2. We found the slow acetylation genotype in 47% of asthmatic subjects and 43% of healthy subjects. Our results suggest that NAT2 slow acetylation is not associated with susceptibility to allergic asthma or severity of disease in our Turkish population.

A large number of studies demonstrated that the NAT2 phenotype measured by the caffeine test may be predicted with high accuracy by genotype. The correlation of genotype with phenotype was at least 90%. The NAT2 structural gene is assigned to chromosome 8, pter-q11, and contains 870 bp open reading frame encoding 290 amino acids. Inheritance via autosomal codominance at the NAT2 locus results in rapid (homozygous), intermediate (heterozygous), and slow (homozygous) acetylators. Two NAT2 alleles are known to code for rapid acetylation, namely NAT2*4 and the rare NAT2*12A. The slow phenotype is encoded by two major slow allelic variants, *5B and *6A, and the less frequent alleles *5A, *5C, and *7B. It is evident there are a number of mutations but only two are required to distinguish the NAT2 phenotypes. Cascorbi et al. reported that the evaluation of only two mutations (C282T and T341C) is sufficient to predict the NAT2 phenotype such as rapid/rapid, rapid/slow, and slow/slow. Positions 282 and 341 comprise all slow acetylator alleles. We therefore used only these two mutations in our study to predict the NAT2 phenotype in Turkish population.

NAT2 represents a bimodal distribution of rapid and slow acetylators with different frequencies in different ethnic populations. We found the slow acetylation genotype in 43% of healthy subjects in our study, this distribution was not much different from the values in other countries. The first study of NAT2 genotyping in the Turkish population has reported that the slow acetylation rate was 57.4% in agreement with the findings of other European studies. However, they sampled only unrelated individuals born and living in south-east Anatolia. Then, Nacak et found the frequency of slow acetylation in 58% of subjects living in the same part of Turkey. The different frequencies of slow acetylases in these studies and ours may be due to different ethnicities of the study populations. They studied only individuals from southeast Anatolia (Asian part of Turkey) and we included individuals living in Istanbul, some of whom had migrated from all parts of Turkey.

Previous studies have indicated that slow acetylation may be associated with allergic diseases. Zielinska et al. reported overrepresentation of phenotypically slow acetylation in children with documented inhalational, food, or mixed allergies. Similarly, Gawronska-Szklarz et al. found that the risk of development of atopic diseases was fivefold greater for homozygous slow acetylators than for healthy subjects. Zielinska and Gawronska-Szklarz recruited subjects from central regions of Poland. Recently, it has been found that homozygous slow acetylation was associated with a predisposition to Ig-E mediated food allergy in Polish children. However, there are no data about whether variance in the NAT2 gene is associated with atopism, especially allergic asthma among other European populations. In our study, we compared NAT2 acetylation genotype of allergic asthmatics and controls and found no significant difference between their slow and rapid acetylation rates. In a recent study, Nacak et al. found increased frequency of slow acetylators among extrinsic asthmatic patients compared with control subjects in southeast part of Turkey. These different results may be due to different population structures. We collected samples from Turks with ethnic origins from all parts of Turkey, not only southeast part of Turkey. People identified as ethnic Turks comprise 80% to 88% of Turkey’s population and include a number of regional groups from the Rumelian Turks to the central Asian Turks. Small number of patients and controls in our study may be another factor for lack of this association.
Our study confirms an inherited mechanism in allergic asthma, with a high prevalence of family history in subjects with asthma as compared with controls (74% versus 25%, respectively). Our negative data indicate the NAT2 genotype, on its own, is not a risk factor for allergic asthma in general Turkish population. Further studies are required to validate these findings.

Acknowledgement

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References

2. Patino CM, Martinez FD. Interactions between genes and environment in the development of asthma. Allergy 2001;56:279-86.

Abbreviation and Acronym List

NA T2: N-acetyl transferase 2
FEV1: Forced expiratory volume at 1 second
PCR: polymerase chain reaction
RFLP:Restriction fragment length polymorphism