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CYP2C19 Gene Polymorphism May Be a Risk Factor for Bronchial Asthma

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Key Words
Asthma · CYP2C19 · Gene polymorphism

Abstract
Objective: The aim of the present study was to investigate whether the genetic polymorphism of CYP2C19 plays a role in susceptibility to bronchial asthma. ***Subjects and Methods:*** 104 healthy individuals who visited our hospital, including hospital staff, and 97 patients with bronchial asthma (62 atopic and 35 nonatopic) participated in this study. CYPC19*2 and CYP21C9*3 alleles were detected by using LightCycler and CYP2C19 mutation detection kits by real-time PCR with LightCycler. ***Results:*** The CYP2C19*3 genotype was found to be the wild type in all cases, and in the control group, the CYP2C19*2 heterozygous genotype had a 2.46-fold increased risk of bronchial asthma compared with the CYPC19*2 homozygous wild genotype in the control group ($p = 0.01$, OR = 2.46, 95% CI 1.24–4.88). ***Conclusion:*** Our data suggest that the CYP2C19*2 heterozygous genotype may be involved in the development of bronchial asthma.

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Introduction

The prevalence of asthma, affecting 300 million people worldwide, has increased over the last decade. Not only the cost, but also the prevalence and mortality have increased, with approximately USD 12 billion in the United States and USD 21.65 billion in Europe per year being spent on medical treatment, hospitalizations, emergency room and office visits. Other 'costs' include the impact of asthma on quality of life, such as restricted activity and decreased productivity from missed work or school [1, 2].

Asthma is a common heterogeneous disease, characterized by reversible airway obstruction and bronchial hyperresponsiveness, usually associated with atopy. The etiology and pathogenesis of asthma remain largely obscure. It is a complex multifactorial disease with an obvious genetic predisposition, hyperreaction failure and the possible involvement of noxious environmental factors [3].

CYP2C19 is a xenobiotic-metabolizing enzyme that metabolizes foreign compounds such as clinically used drugs and other xenobiotics such as environmental chemicals. In the lung, the expression of xenobiotic-metabolizing enzymes such as cytochromes P450 (CYP) and glutathione S-transferases (GST) may be affected by in-

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haled pollutants [4]. Cytochromes P450 are a superfamily of heme proteins that catalyze many types of reactions, predominantly hydroxylation. They participate in the oxidative metabolism of a wide variety of structurally diverse compounds, including endogenously synthesized compounds such as steroids and fatty acids, as well as exogenous compounds such as drugs, carcinogens and environmental agents [5]. In humans, the CYP2C gene subfamily represents a cluster of 4 genes on chromosome 10q24, arranged in the sequential order CYP2C8, CYP2C9, CYP2C19 and CYP2C18 [6]. All members of this subfamily are genetically polymorphic. Clinically, CYP2C19 is the most important among the CYP2C gene subfamily. The predominant genetic polymorphisms in CYP2C19 are 2 variant alleles, CYP2C19*2 and CYP2C19*3, which result in impaired metabolism of CYP2C19 substrates [7, 8]. The substitution of G681A in exon 5 of the CYP2C19*2 variant allele creates an aberrant splice site [7] while the substitution of G636A in exon 4 of the CYP2C19*3 allele results in the emergence of a premature stop codon [8].

It is possible that dietary factors, environmental factors and/or genetic polymorphisms in xenobiotic-metabolizing enzymes may contribute to the development of bronchial asthma. The relationship between genetically determined polymorphic metabolism and susceptibility to allergic diseases has aroused much interest [9–11]. There has been no study regarding the association between asthma and CYP2C19 gene polymorphisms. Therefore, the aim of the present study was to investigate whether or not the genetic polymorphism of CYP2C19 plays a role in susceptibility to bronchial asthma disease.

Subjects and Methods

Subjects

The study population consisted of unrelated white Turkish individuals living in the southern region of Turkey. 104 healthy individuals who visited our hospital for their annual check-up and hospital staff were included as controls, and 97 patients with bronchial asthma (62 atopic and 35 nonatopic) were the subjects who participated in this study. The 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.

An extensive history was taken for all patients and controls and a physical examination, chest radiogram, complete blood count, routine biochemical investigations, skin prick test and pulmonary function tests were conducted. Only patients and controls who had not smoked for 5 years were included in the study.

Table 1. Characteristics of both patients (n = 97) and controls (n = 104)

	Patients	Controls
Age, years	45.51 ± 11.84	49.10 ± 8.65
Sex		
Male	35 (36.0%)	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
Nonatopic	35 (36.1%)	104 (100%)

FEV_1 = Forced expiratory volume in 1 s; FVC = forced vital capacity. * $p < 0.05$, compared with nonatopic asthma.

All patients had both a clinical history of asthma and a positive reversibility test. Asthma was diagnosed according to the American Thoracic Society Statement [9]. All asthmatic subjects were clinically stable and had never experienced exacerbation of symptoms. In addition, they had neither had systemic steroid use, trauma or surgical treatment nor any other signs suggestive of respiratory infection for at least 3 months prior to the study. Pulmonary function tests were performed using V_{max} 22 D (Sensormedics, Calif., USA). Atopy was defined as the presence of a personal history of allergies, seasonal rhinitis, eczema or allergic conjunctivitis. Atopy was further substantiated by a positive skin prick test response to a panel of 13 common aeroallergens (Stallergenes SA, Pasteur, France) during which a skin reaction with a mean wheel diameter of >3 mm larger than that produced with a saline control was observed. The study was approved by the Mersin University Ethics Committee on Human Research and each volunteer gave written informed consent.

DNA Extraction and Genotyping of CYP2C19

Blood was collected in EDTA-containing tubes and DNA was extracted from the leukocytes by a high purity template preparation kit (Roche Diagnostics, GmbH, Mannheim, Germany). The CYPC19*2 and CYP21C9*3 alleles were detected using LightCycler and CYP2C19 mutation detection kits in real-time PCR using a LightCycler instrument (Roche Diagnostics; Catalog No. 3113914).

Statistical Analysis

χ^2 or Fisher's exact test was used to evaluate the distribution of the CYP2C19 genotypes among the asthma patients and control subjects. The association between CYP2C19 genotypes and asthma patients was estimated by computing odds ratios (ORs) and 95% confidence intervals (CIs) from binary logistic regression analyses. All statistical calculations were performed using the SPSS software package version (11.0 for Windows SPSS Inc., Chicago, Ill., USA). All tests were conducted at the $p = 0.05$ level of significance.

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Table 2. Association between atopic and nonatopic asthma with CYP2C19 genotype

Variable	Atopic (n = 62)	Nonatopic (n = 35)	OR
CYP2C19*2 wild	44 (71%)	23 (65.7%)	reference+
CYP2C19*2 heterozygous	18 (29%)	12 (34.3%)	0.78 (0.32–1.90)

Figures in parentheses are 95% CI. + Wild genotypes are used as reference.

Results

The mean age of the patients (± SD) was 45.51 ± 11.84 years and that of the control subjects was 49.10 ± 8.65 years. Other characteristics of patients and controls are given in table 1. The frequencies of CYP2C19 homozygous wild genotypes were 69.1 and 84.6% in patients and controls, respectively. The CYP2C19*2 heterozygous genotypes were more frequent among asthma subjects (30.9%) than among controls (15.4%). Allele frequencies of patients and the control group were in equilibrium with the Hardy-Weinberg equation.

The risk for bronchial asthma was more than 2 times higher (OR = 2.46, 95% CI 1.24–4.88) in individuals with the CYP2C19*2 heterozygous genotype than with the CYP2C19*2 homozygous wild genotype, and this increase was statistically significant (p = 0.01).

With regard to the type of asthma, patients with atopic asthma (63.9%) had a higher rate than nonatopic asthma patients (36.1%) (table 1). There was no risk of atopic asthma in individuals with the CYP2C19*2 heterozygous genotype compared with nonatopic asthma (p = 0.6, OR = 0.78) (table 2). There was no mutant genotype in either CYP2C19 allele in all groups. Also, the CYP2C19*3 heterozygote genotype was not detected in any subject including patients and controls.

Discussion

Several well-known drug-metabolizing enzymes catalyze the activation and detoxification of xenobiotics and are classified as phase I and II enzymes, respectively. Members of the phase I category include cytochrome P450-related enzymes and epoxide hydrolases [9]. CYP2C19 is a xenobiotic-metabolizing enzyme that is involved in the metabolism of several drugs including environmental chemicals [7, 12].

Asthma, as many other multifactorial diseases, results from an interaction between adverse environmental factors and constitutional genetic resistance or susceptibility. The inflammatory process in the bronchi in atopic bronchial asthma stems from interaction of the 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 [13].

In the present study, the CYP2C19*2 heterozygous genotype had a 2.46-fold increased risk of bronchial asthma compared with the control group (p = 0.01, OR = 2.46, 95% CI 1.24–4.88). The associations of xenobiotic-metabolizing enzymes in the development of asthma have been reported previously [11, 13–20]. These xenobiotic-metabolizing enzymes include NAT2, GST, associated with allergic disease and atopy [11, 13], NAT25A, NAT1, NAT2, NAT25A and GST slow acetylator with a risk for asthma [14–20], whereas NAT26A remained unchanged in passive smokers with bronchial asthma [14]. Our finding that CYP2C19 gene polymorphisms may be involved in the development of bronchial asthma confirmed these previous studies [11, 13–20].

Allele frequencies of CYP2C19*2 wild and CYP2C19*2 heterozygous genotypes were 0.93 and 0.07 in the control group, similar to a previous study in which CYP2C19*3 was found to be a wild-type homozygous genotype in the Turkish population [21].

Conclusion

Our results indicate that the CYP2C19*2 heterozygous genotype could be associated with a risk for the development of bronchial asthma. Further studies with a larger study group are needed to confirm this finding.

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