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ORIGINAL RESEARCH

Leukotriene C4 synthase A-444C gene polymorphism in patients with allergic rhinitis

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OBJECTIVE: To determine the frequency of leukotriene C4 synthase A-444C polymorphism in allergic rhinitis patients.

STUDY DESIGN AND SETTING: A prospective, randomized, case-controlled study. Blood samples were obtained from 85 patients with allergic rhinitis and 95 healthy individuals. After the extraction of DNA from the blood samples, the leukotriene C4 synthase A-444C polymorphism was studied by a real-time polymerase chain reaction method.

RESULTS: The AC and CC genotype frequencies were statistically higher in the study group ($P = 0.048$ and $P = 0.037$, respectively). In addition, the AC polymorphism carried an increased risk of developing allergic rhinitis (odds ratio = 2.18, 95% confidence interval, 1.173-4.053, $P = 0.014$).

CONCLUSION: The C allele of the leukotriene C4 synthase gene increases the risk of developing allergic rhinitis.

SIGNIFICANCE: The leukotriene C4 synthase A-444C gene polymorphism is important in susceptibility to allergic rhinitis and this is the first study of this gene polymorphism in allergic rhinitis.

EBM rating: B-3b

Allergic rhinitis (AR) is a common disease, affecting 10% to 25% of the world's population. In the developed countries, the prevalence of AR has increased during the past 3 decades; it is currently estimated at 25% in the general adult population and closer to 40% in children.[1,2] Reasons for this increase are not completely understood; however, it is suggested that environmental and genetic factors are important.[1] Significant investigations have been performed in the area of atopy genetics during the last decade since Cookson et al[3] first reported a linkage between generalized immunoglobulin E (IgE) responsiveness and a DNA polymorphism on chromosome 11q13 in British families.

The allergic reaction is a complex process involving inflammation of the mucous membranes as a result of interaction of inflammatory mediators, which is triggered by an IgE-mediated response to an extrinsic allergen.[4] Leukotrienes (LTs) have important mediator functions in the inflammatory process.[5] The role of LTs on AR have been supported by several lines of evidence.[6,7] LTs are synthesized via the 5-lipoxygenase (5-LO) cascade. The synthesis of LTs is stimulated by an antigen-IgE complex or a cytokine activation of calcium release from activated mast cells and eosinophils.[8,9] The increased intracellular Ca^{++} levels resulted in the activation of the cytosolic phospholipase A_2, which hydrolyzes and releases arachidonic acid from membrane phospholipids.[8,9] Arachidonic acid binds to the 5-LO–activating protein, and it is presented to 5-LO, which forms 5-hydroperoxy-6,8,11,14-eicosatetraenoic acid (5-HPETE) from arachidonic acid and subsequently to LTA4.[8,9] LTA4 can be converted to LTB4 or LTC4 and instantly LTC4 to LTD4. The role of LTB4 in allergic diseases remains unclear.[10] LTA4 is metabolized to LTC4 by the LTC4 synthase enzyme, which is a microsomal glutathione-S-transferase.[8-10] After glutathione conjugation, LTC4 is exported to the blood and hydrolyzed by peptidases to LTD4 and then to LTE4. LTC4, LTD4, and LTE4 are called cysteinyl LTs (CysLTs).[8-10]

CysLTs are bioactive lipids that have been shown to contribute to allergic and inflammatory diseases. It was found that the CysLTs are measurable in nasal secretions

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Table 1
Allelic frequencies of LTC4 synthase A-444C polymorphism in study and control groups

Alleles	Study group (n = 170)	Control group (n = 190)
A	67.6% (115)	80.5% (153)
C	32.4% (55)	19.5% (37)

when nasal mucosa is exposed to an allergen. Many studies showed that the release of CysLTs in nasal lavage was correlated with the familiar symptoms of increased nasal airway resistance, sneezing, and mucous secretion.[6,7,11] LTC4 synthase is expressed in airway inflammatory cells, and it is an integral enzyme that acts as a microsomal glutathione-S-transferase. In 1997, Sanak et al[12] screened the 5'-untranslated region of the LTC4 synthase gene for mutations. They showed an adenine (A) to cytosine (C) transversion, which is found at 444 base pair upstream (−444) from the translation start site.[12] A-444 A/C polymorphism that identified in the LTC4 synthase gene promoter region was predicted to create an extra recognition site for the AP-2 transcription factor and lead to increased gene transcription. Variant C polymorphism is found to be associated with enhanced expression of LTC4 synthase, which results in an increased level of CysLTs.[13] In this study, we aimed to determine the frequency of the LTC4 synthase A-444C gene polymorphism in patients with AR and the risk of developing the disease in patients who have variant alleles.

MATERIALS AND METHODS

This was a hospital-based case-controlled study conducted at the Mersin University Hospital, Mersin, Turkey. The study was designed as a prospective, randomized study. The patients and controls were from the same geographic region (southern Turkey) and of the same ethnic origin (Turkish-Caucasian). All of the participants gave their informed consent, and the study was approved by the institutional ethical committee. AR was diagnosed by means of history, clinical examination, and a skin-prick test. Control subjects were selected among the healthy people with no history of allergies and systemic diseases such as cardiovascular diseases, diabetes mellitus, cancer, chronic obstructive pulmonary disease, hepatitis, and so on.

DNA Extraction and Genotyping of Leukotriene C4 Synthase A-444C

Blood samples were collected in EDTA-containing tubes and stored at +4°C until DNA extraction. DNA was extracted from white blood cells by high pure polymerase chain reaction (PCR) template preparation kit (Cat no: 1 796 828; Roche Diagnostics, GmbH, Mannheim, Germany). Leukotriene C4 synthase A-444C allele was detected by using the leukotriene C4 synthase A-444C toolset for LightCycler (Order no: LTC4S -444–16; Genes-4U AG, Winterthur, Switzerland) and Fast Start DNA Master Hybridization Probe Kit (Cat no: 2 239 272, Roche Diagnostics) by LightCycler 2.0 Real Time PCR. Real-time PCR system performs rapid PCR and simultaneous polymorphism detection by melting curve analysis by monitoring the fluorescence. After amplification of the interested gene by using primers and hybridization probes, a melting curve was generated. Peaks were obtained at 59°C for the C allele and at 65°C for the A allele.

Statistical Analysis

SPSS for Windows (version 10.0; SPSS Inc, Chicago, IL) statistical package software was used for the statistical analysis of the study. Study and control group ages were presented as mean ± SD. The association between LTC4 synthase A-444C polymorphism and the development of AR was examined by the use of logistic regression analysis to calculate the odds ratios (ORs) and 95% confidence interval (CI). A Z test was used for comparison of the heterozygous and homozygous genotype frequencies in the study and control groups. Significance was assumed as $P < 0.05$.

RESULTS

The study consisted of 85 patients with AR (45 women and 40 men) and 95 healthy controls (51 women and 44 men). The mean age was 26 (range, 19-38) in the study group and 27 (range, 18-39) in the healthy controls.

The allelic frequencies for LTC4 synthase polymorphism for wild-type A and variant C were 67.6% and 32.4% in the study group and 80.5% and 19.5% in the controls, respectively (Table 1). The heterozygous and homozygous frequencies within genotype were higher in the study group than in the controls (56.9% and 43.1%, $P = 0.048$ for AC genotype and 70% and 30%, $P = 0.037$ for CC genotype, respectively) (Table 2).

The frequencies of wild-type, heterozygous, and homozygous polymorphic genotypes were 43.5%, 48.2% and

Table 2
LTC4 synthase A-444C homozygous and heterozygous frequencies within genotype in study and control groups

Genotype	Group: Study	Group: Control	*P*
AA	37.8% (n = 37)	62.2% (n = 61)	—
AC	56.9% (n = 41)	43.1% (n = 31)	0.048
CC	70.0% (n = 7)	30.0% (n = 3)	0.037

Table 3
LTC4 synthase A-444C polymorphism and the risk of developing AR

Variable	Study group (n = 85) % (n)	Control group (n = 95) % (n)	OR*	95% CI	*P*
Wild-type (AA)	43.5 (37)	64.2 (61)	1 (reference)	—	—
Heterozygous (AC)	48.2 (41)	32.6 (31)	2.18	1.173-4.053	0.014
Homozygous (CC)	8.3 (7)	3.2 (3)	3.847	0.937-15.801	0.062

*From conditional logistic regression. Wild-type genotype is used as reference.

8.3% in the study group, and 64.2%, 32.6%, and 3.2% in the controls, respectively. The AC polymorphism carried an increased risk of developing AR (OR = 2.18, 95% CI, 1.173-4.053, $P = 0.014$), whereas the CC polymorphism did not (OR = 3.847, 95% CI, 0.937-15.801, $P = 0.062$) (Table 3).

DISCUSSION

We investigated whether the genetic polymorphism of LTC4 synthase A-444C plays a role in the susceptibility to AR or not. We found a significant increased risk of developing AR in subjects who had AC genotype for LTC4 synthase gene promoter region. Additionally, CC genotype frequency was significantly higher than the control subjects; however, in the risk analysis, the *P* value for CC genotype was not less than 0.05 because of the low number of the homozygous cases in both groups. To our knowledge, this is the first report in the English literature about the association between LTC4 synthase gene polymorphism and AR.

The effects of CysLTs on the lower-respiratory system in asthmatic patients have been studied previously. Sayers et al[14] showed the association between the A-444C allele and bronchial hyperresponsiveness to metacholine. Also, they showed that asthmatic children with this polymorphism had a lower mean basal forced expiratory volume in 1 second. However, they concluded that this polymorphism does not support the susceptibility to asthma. In the study presented here, a statistically significant association between LTC4 A-444C polymorphism and susceptibility to AR was found. Sanak et al[12] reported that the frequency of the C allele was greater in aspirin-induced asthma. They suggested that the C allele of this single polymorphism was responsible for a gain in function of the regulatory elements of this gene, which, in turn, resulted in increased transcription of the LTC4 synthase gene. In contrast, Bigby[10] showed that the polymorphism of the same nucleotide in the promoter region of the LTC4 synthase gene was associated with decreased expression of the enzyme. According to the results of the present study, it might be hypothesized that LTC4 synthase gene polymorphism enhances the expression of this gene, and then this leads to an increase in the production of CysLTs. Supporting this hypothesis, Sanak et al[12] also showed that peripheral blood eosinophils obtained from asthmatic patients have increased messenger RNA encoding for LTC4 synthase.

CONCLUSION

Allergic rhinitis is a complex disease affected by interacting genetic and environmental factors. It is clear that no single genetic risk factor could be responsible for the development of the disease. However, the approach "epidemiology of genetic features" may help the detection and prevention of high-risk in individuals for AR. In this population, we found a significantly increased risk of developing AR in subjects who had AC genotype for LTC4 synthase gene promoter region. Our findings need additional studies with CysLTs analysis in the nasal lavage fluids of the patients who have LTC4 synthase gene polymorphism and pharmacological studies using CysLT synthesis inhibitors or receptor antagonists. Further studies should also be undertaken on a larger sample size for evaluating the exact importance of this gene polymorphism in the susceptibility to AR.

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