Original article

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TRANSFORMING GROWTH FACTOR B1 C-509T GENE POLYMORPHISM IN PATIENTS WITH BRONCHIAL ASTHMA

Milena Despotović[1], Tatjana Jevtović-Stoimenov[1], Ivana Stanković[2,3], Dušica Pavlović[1], Branka Đorđević[1], Jelena Bašić[1], Andrej Veljković[1]

Bronchial asthma is a poligenic disorder caused by the influence of genetic and envioromental factors. The functional single nucleotide polymorphism (SNPs) in the regulatory regions of the cytokine genes may affect the cytokine production and thus play a contributory role in the pathogenesis of asthma. Substitution of cytosine (C) by thymine (T) at the position -509 in the promoter region of the transforming growth factor β1 (TGF-β1) gene could be associated with asthma.

The aim of this study was to investigate the association of the TGF-β1 C-509T polymorphism with asthma and to determine the distribution of this polymorphism in the Serbian population.

A total of 57 patients with diagnosed asthma and 49 healthy controls were screened for TGF-β1 C-509T polymorphisms using the polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) method.

The TGF-β1 C-509T genotype (p=0.413) and allele frequencies (p=0.227) distributions in patients did not reveal statistically significant difference compared to controls. Additionally, no difference in genotype and allele frequencies distribution between male and female subjects was observed.

In conclusion, to the best knowledge of the authors, this is the first study examining the association of TGF-β1 C-509T polymorphism in the Serbian patients with asthma. The present study did not confirm the specific role of TGF-β1 C-509T polymorphisms in asthma. No differences in the distribution of TGF-β1 C-509T polymorphism between patients and healthy subjects were observed. *Acta Medica Medianae 2014; 53(4):22-26.*

***Key words**: bronchial asthma, transforming growth factor-β1 (TGF-β1), gene polymorphism*

University of Niš, Faculty of Medicine, Department of Biochemistry, Serbia[1]
University of Niš, Clinical Centre Niš, Clinic for Pulmonary Diseases and Tuberculosis Knez Selo, Serbia[2]
University of Niš, Faculty of Medicine[3], Serbia

Contact: Milena Despotović
Faculty of Medicine, Department of Biochemistry
Bul. Zorana Đinđića 81, 18000 Niš, Serbia
E-mail: milena.despotovic@ymail.com

Introduction

Bronchial asthma is a chronic inflammatory disease of the airways characterized by the recurrent episodes of wheezing, dyspnea, cough and brethlessness. The pathophisiology of bronchial asthma is not entirely understood. It is considered to be a complex polygenic disorder where both genetic and enviromental factors are resposible for the occurence and evolution of disease (1). Family-based studies suggest that genetic factors could influence asthma-related phenotypes and control the susceptibility of the airways to inflammation (2). The inflammatory process in asthma is mediated by a complex network of cytokines which are involved in managing the chronic inflammation by recruiting, activating and promoting the survival of multiple inflammatory cells in the respiratory tract (3).

The transforming growth factor β1 (TGF-β1) is a member of the large family of poly-peptide growth factors named TGF-β family. It is a pleotropic cytokine which shows both pro- and anti-immflamatory effects. The TGF-β1 is produced by the number of cells in the airways, including macrophages, epithelial cells, fibroblasts, and eosinophils (4). Other cytokines involved in asthma pathogenesis, including IL-5, IL-13 as well as TGF-β itself, can induce its production and release from these cells (5). The TGF-β1 has an important role in growth, development, transformation, tissue repair, fibrosis and modulation of inflammatory immune response (6). The TGF-β1 was shown to be involved in epithelial changes, subepithelial fibrosis, airway smooth muscle remodeling, and microvascular changes (7).

Genetic variations within the cytokine genes may be important for understanding indivi-

dual predisposition and susceptibility to different clinical conditions. The substitution of cytosine (C) by thymine (T) at the position -509 in the promoter region of the TGF-β1 gene is associated with enhanced TGF-β1 transcription and increased TGF-β1 plasma concentrations (8). However, previous studies examining the relevance of TGF-β1 promoter single nucleotide polymorphism (SNP) in asthma have shown controversial results. To the best of our knowledge, there are no available studies of TGF-β1 C-509T polymorphism in the Serbian patients with asthma. Thus, the main objective of this study was to investigate the association of the TGF-β1 C-509T polymorphism with asthma and to determine the distribution of this polymorphism among the Serbian population.

Patients and methods

One hundred and six subjects, i.e. 57 patients with diagnosed bronchial asthma and 49 unrelated, healthy subjects, were involved in this study. Bronchial asthma was diagnosed according to the guidelines of the Global Initiative for Asthma at the Clinic for Pulmonary Diseases and Tuberculosis, Clinical Center Nis, Serbia. Control subjects had no previous history of asthma, atopy, other allergic, or acute and chronic inflammatory diseases. An informed consent was obtained from all subjects prior to the study entry according to the declaration of Helsinki. The study was approved by the Ethical Committee of the Faculty of Medicine, University of Nis, Serbia.

DNA isolation and SNP detection

Two-hundred microliters of venous blood samples were used for genomic DNA isolation by QIAamp DNA Blood Mini Kit (Quiagen GmbH, Hilden, Germany). Biallelic TGF C-509T polymorphism was determined using polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) technique. The PCR was performed in a final volume of 25μl containing 20ng of DNA, 12.5 μl KAPA2G Fast HotStart ReadyMix (Kapa Biosystems, Inc, USA) and 20 pmol of each primer (F 5'-GGA GAG CAA TTC TTA CAG GTG-3', R 5'-TAG GAG AAG GAG GGT CTG TC-3'). The PCR conditions were as follows: initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 95°C for 15s, annealing at 60°C for 15s, and extension at 72°C for 15s. The reaction was ended with the final extension at 72°C for 30s. After the determination of PCR products by 2% agarose gel electrophoresis, the amplification products were digested using HpyF3I (Fermentas GmbH, St. Leon-Rot, Germany) overnight at 37°C. Restriction fragments were analyzed by 8% polyacrylamide gel electrophoresis.

The results' interpretation was as follows: the presence of TGF -509T allele was represented by 120 bp product, while the presence of TGF -509C allele was detected as 74 and 46 bp fragments (Figure 1.).

Statistical analysis

Differences in genotype and allele frequencies between patients and controls were determined using the chi-square (χ^2) test or two-tailed Fisher's test when the number of expected cases was small. The odds ratio (OR) and 95% confidence interval (95% CI) were calculated to estimate the strength of the association. The differences were considered significant at $p<0.05$. Statistical analyses were performed usingthe SPSS version 13.0 statistical package (SPSS Inc., Chicago, IL, USA).

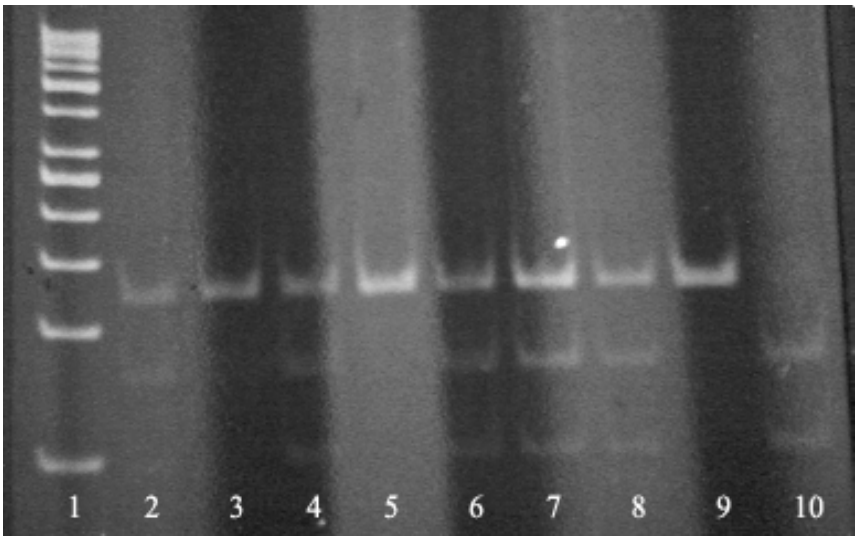

Figure 1. 8% polyacrylamide gel electrophoresis of TGF-β1 C-509T polymorphism
Lane 1 – ladder; lanes 2, 4, 6, 7, 8 – CT; lane 3, 5, 9 – TT; lane 10 – CC

Results

Fifty-seven patients with asthma, 34 women (59.65%) and 23 men (40.35%) with the mean age at the study entry 49.71±13.94 (range 21-74 years) were involved in this study. In the control group, consisting of 49 healthy, unrelated individuals (mean age at the study entry 49.00 ±16.73, range 21-79), there were 28 women (57.14%) and 21 men (42.86%).

Genotype frequencies for the SNPs in the study groups were in Hardy–Weinberg equilibrium ($p>0.05$).

Distribution of the TGF-β1 C-509T genotypes was as follows: CC genotype was present in 35.1% of asthmatics, CT in 54.5% and TT in 10.5%, while 26.5% of control subjects had CC genotype, 55.1% CT and 18.4% TT. The observed genotype distribution of TGF-β1 C-509T polymorphism did not show significant differences in patients compared to controls (Table 1.).

Moreover, no differences in the distribution of TGF -509C and TGF -509T alleles between patients and controls were observed (Table 2.). Table 3 represents the TGF-β1 C-509T genotype distribution and allele frequencies in female and male subjects. After the stratification of the samples by gender, no differences in genotype or allele frequency distribution were observed ($p>0.05$).

Discussion

The SNPs C-988A, G-800A and C-509T have recently been identified in the promoter region of the TGF-β1 gene. The functional SNPs in the regulatory region of the cytokine genes may affect cytokine production and thus play a contributory role in the pathogenesis of asthma.

The TGF-β1 C-509T polymorphism (rs-1800469) is widely studied in asthma, but the results still remain inconclusive. Genetic variants that are associated with the increased TGF-β1 activity, such as C to T base exchange at the position-509 of TGF-β1 gene, are expected to be associated with higher asthma prevalence or increased asthma severity (9).

The distribution of polymorphic TGF-β1 -509TT genotype varies among healthy subjects in different populations. In Caucasians TGF-β1 -509TT genotype is presented in 6.5% to 16.5%, while in Asian population it varies between 5% and 33% (10). Similar to the results obtained in Caucasian population, we found this genotype to be present in 18.4% of healthy Serbian individuals.

The association of TGF-β1 C-509T polymorphism with asthma has been reported in several previous studies (1.9-12). However, our study failed to confirm the association between TGF-β1 C-509T polymorphism and asthma in the Serbian patients. Our results are in accordance with those obtained in Czech and Polish adult population and German pediatric population (13-15). Also, Kumar et al. reported that TGF-β1 C-509T polymorphism itself is not associated with asthma in Asian patients (16).

The meta-analysis by Zhang et al. showed a significant prevalence of the TGF-β1-509T al-lele in severe asthma, and also suggested that the carriers of the TGF-β1 -509T allele have a nearly 36% increased risk of asthma predomi-nantly in the Asian population (10). The associ-ation of polymorphic TGF-β1 -509T allele with asthma is confirmed in several other studies as well (1,9,17). On the contrary, our study did not show significant differences in allele frequency distributions between patients and controls.

Table 1. Distribution of TGF-β1 C-509T genotypes in patients with asthma and controls

Genotype	Asthma n=57 (%)	Control n=49 (%)	χ^2	*p*
CC	20 (35.1)	13 (26.5)	1.767	0.413
CT	31 (54.4)	27 (55.1)		
TT	6 (10.5)	9 (18.4)		
CC	20 (35.1)	13 (26.5)	1.497	0.343
CT+TT	37 (64.9)	36 (73.5)		

n = number of study subjects

Table 2. Allele frequencies of TGF-β1 C-509T genotypes in patients with asthma and controls

Allele	Asthma n (%)	Control n (%)	χ^2	*p*	OR	95% CI
C	71 (62.3)	53 (54.1)	1.459	0.227	1.402	0.81-2.43
T	43 (37.7)	45 (45.9)				

n = number of study subjects, OR = odds ratio, CI = confidence interval

Additionally, no differences in genotype and allele frequencies distribution among the genders are observed.

Discrepancy in the results presented in previous studies may be due to different ethnicity and heterogeneity of study groups, as well as specific gene-gene and gene-environment interactions. Moreover, since the cytokines involved in asthma pathogenesis act in a highly complex coordinated network, it would be important to investigate the common influence of the functional genetic polymorphisms which regulate their production.

Conclusion

This is the first study examining the association of TGF-β1 C-509T polymorphism in Serbian patients with asthma. The present study did not confirm the specific role of TGF-β1 C-509T polymorphisms in asthma. No differences in the distribution of TGF-β1 C-509T polymorphism between patients and healthy subjects were observed.

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POLIMORFIZAM GENA ZA TRANSFORMIŠUĆI FAKTOR RASTA B1 C-509T KOD OBOLELIH OD BRONHIJALNE ASTME

Milena Despotović, Tatjana Jevtović-Stoimenov, Ivana Stanković, Dušica Pavlović, Branka Đorđević, Jelena Bašić, Andrej Veljković

Bronhijalna astma je poligensko oboljenje uzrokovano uticajem genetskih faktora i faktora spoljašnje sredine. Funkcionalni polimorfizmi nukleotidne sekvence (SNP) u regulatornim regionima gena za citokine mogu uticati na produkciju citokina, a samim tim imati i ključnu ulogu u patogenezi astme. Zamena citozina (C) timinom (T) na poziciji -509 u promotornom regionu gena za transformišući faktor rasta β1 (TGF-β1) može biti udružena sa astmom.

Cilj ove studije bio je da se utvrdi povezanost TGF-β1 C-509T polimorfizma sa bronhijalnom astmom, kao i distribuciju ovog polimorfizma u srpskoj populaciji.

Genski polimorfizam TGF-β1 C-509T određivan je kod 57 bolesnika sa astmom i kod 49 zdravih osoba korišćenjem lančane reakcije polimeraze, praćene metodom polimorfizama dužine restrikcionih fragmenata (PCR-RFLP).

Distribucija TGF-β1 C-509T genotipa (p=0.413) i frekvencije alela (p=0.227) nisu pokazale statistički značajne razlike između bolesnika i kontrolne grupe. Takođe, nije bilo razlike u distribuciji genotipa, niti u distribuciji frekvencije alela između muškog i ženskog pola.

Prema saznanju autora, ovo je prva studija kojom je ispitivana povezanost TGF-β1 C-509T genskog polimorfizma sa astmom u srpskoj populaciji. Rezultati naše studije nisu potvrdili povezanost TGF-β1 C-509T polimorfizma sa bronhijalnom astmom. *Acta Medica Medianae 2014;53(4):22-26.*

***Ključne reči**: bronhijalna astma, transformišući faktor rasta β1 (TGF-β1), genski polimorfizam*