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KLİNİK ÇALIŞMA
RESEARCH ARTICLE

Lack of Association of 1513 A/C Polymorphism in P2X₇ Gene with Susceptibility to Pulmonary and Extrapulmonary Tuberculosis

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SUMMARY

Lack of Association of 1513 A/C Polymorphism in P2X₇ Gene with Susceptibility to Pulmonary and Extrapulmonary Tuberculosis

Introduction: Tuberculosis, is one of the a leading causes of death worldwide, is characterized by different clinical forms including: latent, localized pulmonary infection and extrapulmonary tuberculosis. Candidate gene association studies have implicated common polymorphisms in genes that may influence the development of tuberculosis. This study, aimed to elucidate the role of P2X₇ gene in 1513A/C polymorphism the etiopathogenesis of tuberculosis.

Materials and Methods: The study included 160 patients with tuberculosis (71 pulmonary and 89 extrapulmonary tuberculosis) and 160 healthy controls. Genomic DNA was isolated and 1513A/C polymorphism in P2X₇ gene was genotyped by PCR-RFLP method.

Results: Frequency of P2X₇ AA genotype was 47.5% in controls and 56.87% in patients, AC frequency was 39.37% controls and 32.5% in patients, CC genotype was 13.12% in controls and 10.62% in patients. No significant difference in allele and genotype frequencies (1513A/C polymorphism) between tuberculosis patients and controls was found.

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Conclusion: The results suggest that 1513A/C polymorphism of P2X₇ gene is not associated with pulmonary and extrapulmonary tuberculosis in the Eastern Turkey.

Key words: Tuberculosis, P2X₇, polymorphism

ÖZET

Pulmoner ve Ekstrapulmoner Tüberkülozlu Hastalarda P2X₇ Genindeki 1513 A/C Polimorfizmin Hastalıkla İlişkisinin Olmaması

Giriş: Latent, pulmoner ve ekstrapulmoner şeklinde farklı klinik formları olan tüberküloz, dünya genelinde ölüme sebebiyet veren hastalıkların en önemlilerinden biridir. Aday genlerle yapılan çalışmalarda yaygın polimorfizmlerin tüberkülozun gelişmesinde etkili olabileceği sonucuna varılmıştır. Bu çalışmada P2X₇ genindeki 1513 A/C polimorfizminin tüberkülozun etyopatogenezindeki rolünün ortaya çıkarılması amaçlanmıştır.

Materyal ve Metod: Bu çalışma 160 tüberkülozlu (71 pulmoner tüberkülozlu ve 89 ekstrapulmoner tüberkülozlu) 160 da sağlıklı bireyi kapsamaktadır. Genomik DNA izolasyonu gerçekleştirildikten sonra P2X₇ genindeki 1513 A/C polimorfizmi PCR-RFLP metoduyla genotiplendirildi.

Bulgular: AA genotipinin frekansı kontrol grubunda %47.5, hastalarda %56.87, AC genotipinin frekansı kontrol grubunda %39.37, hastalarda %32.5, CC genotipinin frekansı kontrol grubunda %13.12, hastalarda %10.62 olarak saptandı. Hastalar ile kontrol grubunun allel ve genotip frekansları arasında anlamlı bir farklılık bulunamadı.

Sonuç: Sonuçlarımız Türkiye'nin doğu bölgesinde P2X₇ genindeki, 1513 A/C polimorfizminin pulmoner ve ekstrapulmoner tüberküloz hastalığıyla ilişkisinin olmadığını gösterdi.

Anahtar kelimeler: Tüberküloz, P2X₇, polimorfizm

INTRODUCTION

Tuberculosis is a major cause of morbidity and mortality worldwide, especially in Asia and Africa. Genetic variability, combined with environmental factors, are shown to contribute to the risk of developing active tuberculosis (1). Some patients have identifiable risk factors such as diabetes mellitus, malnutrition, human immunodeficiency virus (HIV) infection or immunosuppressive therapy; however many patients exhibit none of these clinical risk factors (2).

Some genetic variations such as Mendelian-inherited mutations in the genes encoding interferon-gamma (IFN- γ), interleukin-12, and signal transducers are rare and are associated with severe mycobacterial infection. Recently some researchers have reported that polymorphism in IFN- γ and MCP-1 genes were associated with an increased risk of developing active tuberculosis in some Tunisian patients (3). Other genetic variations such as polymorphisms in the genes encoding human leukocyte antigen (HLA), P2X₇ receptor, the solute carrier family 11 member a1 protein (SLC11A1, formerly known as NRAMP1), and vitamin D3 receptor (VDR), which occur more commonly, are considered to account for the susceptibility of the general population to tuberculosis (4,5).

Human P2X₇, which encodes the P2X₇ receptor, has been cloned and mapped to the human chromosome 12q24 and linked to tuberculosis susceptibility (6).

P2X₇ is highly polymorphic and several single nucleotide polymorphisms (SNPs) that lead to the loss of receptor function have been identified (7,8). The most common is the 1513A \rightarrow C polymorphism because glutamic acid at position 496 changes to alanine. The function of the P2X₇ receptor in macrophages from subjects homozygous for the 1513C allele is ablated, and the function of the P2X₇ receptor in macrophages from heterozygous subjects is significantly impaired.

The aim of this study was to determine the genotype and allelic distribution and possible link to susceptibility to tuberculosis of P2X₇ 1513A \rightarrow C polymorphism in the Eastern Turkey.

MATERIALS and METHODS

Patients and Control Group

The study was approved by the ethics committee of Firat University Medical Faculty. A total of 160 patients with active tuberculosis (71 pulmonary tuberculosis, 89 extrapulmonary tuberculosis patients; with mean age 37.43 \pm 14.58 years) were recruited from those who were treated and followed up in the Pulmonary Diseases Department of the Firat University Hospital, Turkey. As healthy controls, age, sex and origin-matched 160 unrelated selected healthy subjects (mean age 39.27 \pm 13.84 years).

Patients were recruited between September 2009 and May 2011 and were selected after confirmation of the

infection according to the criteria defined by the American Thoracic Society (9). The diagnosis of active pulmonary tuberculosis was based on clinical examination and the presence of the *Mycobacterium tuberculosis* strain in sputum smears and cultures on Lowenstein-Jensen and Coletsos media. Active extrapulmonary tuberculosis was identified by histological examination (granulomatous formations) of data and confirmed by conventional bacteriological methods.

Genotype Analysis

Peripheral blood samples were drawn from all of the participants, and were taken into tubes containing ethylenediamine tetraacetate (EDTA) and DNA was extracted and stored at -20°C until analysis of the P2X₇ polymorphism.

Genomic DNA was prepared from 300 µL of fresh blood peripheral blood mononuclear cells using a Wizard Genomic DNA Purification Kit (Promega Corporation, Madison, WI) according to the manufacturer's recommendations. Afterwards the samples were quantified using a Nanodrop spectrophotometer (UV-Visible NanoDrop 1000, Thermo Fisher Scientific Inc.) and standardized to 50 ng µL⁻¹. Aliquots were stored at -20°C for further genotyping.

The 1513A→C SNP was genotyped by PCR-restriction fragment length polymorphism (RFLP) with the following primers: 5'-AGA CCT GCG ATG GAC TTC ACA G-3' (forward) and 5'-AGC GCC AGC AAG GG CTC-3' (reverse) (10). The PCR conditions were: initial denaturation at 95°C for 5 minutes; 36 cycles of 95°C for 30 seconds, 36 cycles of 66.3°C for 30 seconds, 36 cycles of 72°C for 40 seconds, and final elongation at 72°C for 7 minutes. The PCR products were digested at 37°C for 4 hours with 5.0 U of HaeIII (Promega). The digested products were run on a 3% agarose gel and visualized with 10 ng/mL ethidium bromide.

Statistical Analysis

Statistical analyses were carried out using SPSS software version 16 (SPSS Inc. Chicago IL USA). The genotype distribution was tested for Hardy-weinberg equilibrium using chi-square test in tuberculosis patients and controls. The distributions of P2X₇ polymorphism between tuberculosis patients and healthy controls were compared using the Fisher's exact test. $p < 0.05$ was considered significant.

RESULTS and DISCUSSION

Genotypes and alleles frequencies of the 1513A→C polymorphism of P2X₇ gene in tuberculosis patients and controls are shown in the Table 1.

The frequency of the 1513A allele in the tuberculosis patients was 0.27, where as that of 1513C was 0.73, and no significant differences were noted in comparison with the frequencies in the case of the control subjects ($p > 0.05$, Table 1). Analysis of genotypic distribution using chi-square test revealed no significant difference between the two groups ($p > 0.05$, Table 1). Moreover, no significant associations were found between the genotypic or the allelic distributions and pulmonary or extrapulmonary tuberculosis.

This study was undertaken to gain insight into the role of the human P2X₇ receptor gene in the susceptibility to tuberculosis in the Eastern Turkey. Areas of inflamed tissue such as tuberculosis granulomata contain numerous monocytes, macrophages and high local concentrations of ATP (released from dying and activated cells) and pro-inflammatory cytokines (11). It is possible that the ATP- linked P2X₇ effector pathway may contribute to host immunity of *M. tuberculosis*.

There is substantial evidence that show host genetic factors are important in determining susceptibility to mycobacteria (12,13). To the best of our knowledge, this is the first study investigating the association between P2X₇ genetic polymorphisms and the sus-

Table 1. Genotype and allele frequency of the 1513A→C polymorphism of P2X₇ in patients and controls

Subjects (n)	Genotype				Allele			HWE (P)*
	AA (%)	AC(%)	CC(%)	p	A (%)	C (%)	p	
Control (160)	76 (47.50%)	63 (39.37%)	21 (13.12%)		67	33		0.176
Patients (160)	91 (56.87%)	52 (32.5%)	17 (10.62%)	0.244	27	73	0.10	0.028
Pulmonary TB (71)	44 (61.97%)	18 (25.35%)	9 (12.67%)	0.093	75	25	0.10	0.0053
Extrapulmonary TB (89)	47 (52.80%)	34 (38.20%)	8 (8.98%)	0.553	28	72	0.275	0.607

TB: Tuberculosis * HWE (P) is the significance of correspondence to Hardy-Weinberg proportions according to chi-square test.

ceptibility to pulmonary and extrapulmonary tuberculosis in the Eastern Turkey.

Several SNPs in the P2X₇ gene that affect the function of this receptor have been described. The more relevant SNPs of P2X₇ reported correspond to the 1513A/C that affects the carboxy terminal tail and leads to a loss of receptor function as assessed by ATP induced Ca²⁺ ethidium bromide influx and IL-1 β release (14-18). Previous studies have shown that macrophages from individuals homozygous for the 1513C loss of function allele have complete loss of receptor function while individual heterozygous for this polymorphism still maintain 50% function to kill intracellular parasites such as *M. tuberculosis* or *T. gondii* after exposure to ATP compared with macrophages from individuals with the 1513A wild type allele (17,18).

This study indicates that there is no risk between 1513A/C polymorphism in the P2X₇ gene and tuberculosis disease in the Eastern Turkey. These results are in agreement with previously reported data in an Australian Vietnamese population and a Chinese Han population (17,19). It was reported in other studies in Mexicans, Russians and Tunisians an association was found between the 1513A/C polymorphism and susceptibility to active tuberculosis (10,20,21).

The heterogeneity of the results reported by these studies could be related to one or more of the following parameters: (a) the ethnic origin of patients. For example, it has been reported that ethnic-specific genetic variations may influence host immunity to tuberculosis, causing different tuberculosis susceptibilities (22). In this setting, among control subjects, the prevalence of the 1513C loss of function allele was 7.6% in a Gambian population, 13% in a Russian Slavic population, 17% in a Tunisian population, 19% in a Mexican population, 20.6% in a Vietnamese population and 33% in Turkish population (the present study); (b) Sample size and study design which may affect statistical calculations; (c) As genetic susceptibility to tuberculosis is polygenic, the other described functional SNPs occurring in the P2X₇ gene may be associated with susceptibility to active tuberculosis (10,17,20-23). Further studies are needed to investigate whether this functional polymorphism is associated with a risk of developing active tuberculosis.

Our results indicate that the 1513A/C polymorphism of P2X₇ are not associated with an increased susceptibility to *M. tuberculosis* infection in this population. Because host susceptibility to tuberculosis is likely to

be under polygenic control, and the risks attributable to each polymorphism is modest, the precise mechanism(s) of underlying susceptibility or protection, as well as its possible clinical relevance, remains an interesting topic to be explored further.

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CONFLICT of INTEREST

None declared.

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