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IL-1 β polymorphism in COPD patients in Turkish population

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SUMMARY

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Introduction: Chronic obstructive pulmonary disease (COPD) is a common respiratory condition characterized by persistent airflow limitation and is associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases. Interleukin-1 beta (IL-1 β) is a major pro-inflammatory cytokine expressed by many cells such as macrophages, neutrophils and monocytes and functions in cellular activities such as proliferation, differentiation and apoptosis. Recent studies demonstrate controversial results about the relationship between IL-1 β and COPD. The aim of this study is to investigate the association between IL-1 β -511 (rs 16944) and +3954 (rs 1143634) gene polymorphisms and COPD in Turkish population.

Patients and Methods: A total of 152 subjects were recruited in the study and divided into three groups: 72 COPD patients, 41 healthy smokers and 39 never-smokers. PCR-RFLP method was used to determine the allele frequencies, genotype and haplotype distributions.

Results: We did not find any significant difference between the gene polymorphisms and COPD by means of genotype frequencies, haplotype association, stage, gender or smoking status ($p < 0.05$).

Conclusion: Our results do not show any evidence of association between COPD and IL-1 β -511 and +3954 gene polymorphisms in Turkish population.

Key words: COPD, IL-1 β -511 and +3954, polymorphism

ÖZET

Türk popülasyonundaki KOAH hastalarında IL-1 β polimorfizmi

Giriş: Kronik obstrüktif akciğer hastalığı (KOAH) persistan hava akım kısıtlanması ile karakterize sık görülen bir hastalıktır. Çeşitli zararlı gaz ve partiküller hava yollarında kronik inflamasyona neden olurlar. İnterlökin-1 beta (IL-1 β), makrofajlar, nötrofiller ve monositler gibi birçok hücre tarafından üretilen ana pro-

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inflamatuvar sitokindir ve proliferasyon, farklılaşma ve apoptoz gibi hücrel aktivitelere görev alır. Daha önce yapılmış olan ve IL-1 β ve KOAH arasındaki ilişkiyi inceleyen çalışmalarda çelişkili sonuçlar bulunmuştur. Bu çalışmanın amacı Türk popülasyonunda IL-1 β -511 (rs 16944) ve +3954 (rs 1143634) gen polimorfizmleri ve KOAH arasındaki ilişkiyi incelemektir.

Hastalar ve Metod: Çalışmaya toplam 152 hasta dahil edilmiş ve üç gruba bölünmüştür; 72 KOAH hastası, 41 sigara içen sağlıklı birey ve 39 hiç sigara içmemiş sağlıklı birey. Polimeraz zincir reaksiyonu-restriksiyon parça uzunluk polimorfizmi (PZR-RFLP) yöntemi kullanılarak allel frekansları, genotip ve haplotip dağılımı belirlenmiştir.

Bulgular: Genotip frekansları, haplotip ilişkisi, evre, cinsiyet veya sigara içme durumları açısından incelendiğinde gen polimorfizmleri ve KOAH arasında bir ilişki saptayamadık ($p < 0.05$).

Sonuç: Sonuçlarımız KOAH ve IL-1 β -511 ve +3954 gen polimorfizmleri arasında bir birliktelik olduğuna dair bir kanıt ortaya koymamaktadır.

Anahtar kelimeler: KOAH, IL-1 β -511 ve +3954, polimorfizm

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is defined as a common, preventable but incurable disease that is characterized by persistent respiratory symptoms and airflow limitation due to a chronic inflammatory response in lung to noxious particles or gases. It is composed of a spectrum varying between small airways disease and parenchymal destruction (1). COPD is a major cause of chronic morbidity and mortality around the world (2-4).

The major risk factor for COPD is cigarette smoking. In spite of this fact only 10-20% of heavy smokers develop COPD (5). This suggests that beyond environmental factors also genetic risk factors may play role in the development of the disease.

According to case-control studies, prevalence of COPD is increased in relatives and also lung function is also more correlated between parents and children than non-relatives suggesting a strong genetic basis for COPD. Twin studies provide a more accurate data supporting a genetic basis for disease susceptibility (6,7).

To date α 1-AT deficiency remains the only proven genetic risk factor for COPD. In COPD, it is likely that multiple genes may play role and the genetic susceptibility may depend on several gene polymorphisms acting together (8-10). Possible candidate genes involved in the pathogenesis of COPD, act in the production of proteases and antiproteases, mucociliary clearance, antioxidant metabolism, airway hyperresponsiveness and inflammatory response (7,11,12). Inflammatory mediators such as the Vitamin D-binding protein, Tumor Necrosis Factor- α IL-1 complex affect genetic susceptibility (9,12).

The IL-1 family consists of 11 pro-inflammatory cytokines, including IL-1 α and IL-1 β and a naturally occurring anti-inflammatory agent, the IL-1 receptor antagonist (IL-1RN). IL-1 β is a major actor in many cellular activities including apoptosis, inflammatory response and cell differentiation. IL-1 β -511 C/T (rs 16944) and +3954 C/T (rs 1143634) polymorphisms were studied in relation to COPD and asthma in different populations and contradictory results have been reported (13-17).

In our study we investigated the IL-1 β -511 C/T and +3954 C/T polymorphisms and haplotype frequencies in Turkish COPD patients and control subjects.

PATIENTS and METHODS

Blood samples were collected from 72 patients (62 males and 10 females) with COPD (all smokers COPD group), 41 healthy smokers and 39 healthy non-smokers. The confirmative diagnosis of COPD was based on the subject's medical history, physical examination and pulmonary function tests, using Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines (1). All patients had moderate-to-very severe COPD according to the GOLD classification of severity. Patients with post-bronchodilator FEV₁ values of $\geq 80\%$ were excluded from this study. All patients gave written informed consent and the studies were performed according to the Declaration of Helsinki. The study was approved by the Ethics Committee of Istanbul University Cerrahpaşa Medical Faculty.

Genomic DNA for molecular analysis was isolated from peripheral blood samples by Proteinase K digestion and a salting-out with ammonium acetate. Polymerase chain reactions (PCR) to amplify a segment of the IL-1 β gene including the polymorphic sites IL-1 β -511 C/T (rs 16944) and +3954 C/T (rs

1143634) were performed separately with 10 pmol forward -5' TGG CAT TGA TCT GGT TCA TC-3'; reverse -5' GTT TAG GAA TCT TCC CAC TT -3', and forward -5' GTT GTC ATC AGA CTT TGA CC -3'; reverse -5' TTC AGT TCA TAT GGA CCA GA -3' primers, respectively in separate mixtures (25 μ l) containing 0.2 mM dNTP, 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 0.08% Nonidet P40, 2.5 mM MgCl₂, 1 U Taq polymerase (MBI, Fermentas, Lithuania) and approximately 50ng genomic DNA. The mixture was heated to 95°C for 10 min and then subjected to 36 cycles of 94°C for 45 s, 54°C for 50 s and 72°C for 1 min. The final extension was carried out for 5 min at 72°C.

The PCR products were digested separately with restriction enzymes Aval (for IL-1 β -511 C/T polymorphism) at 37°C overnight. Digestion products were electrophoresed on a 2 % agarose gel in a 0.5 x TBE buffer at 120 V for 1 hour. The genotypes were determined under UV illumination using a video gel documentation system (Vilber-Lourmat, Cedex, France) after staining with ethidium bromide.

Genotypes were evaluated by the following pattern: Single band of 304 bp: TT homozygote, two bands of 190 and 114 bp: CC homozygote, three bands of 304, 190 and 114 bp: CT heterozygote and TaqI (for IL-1 β +3954 C/T polymorphism) at 56°C for 3 hours generating the following pattern: Single band of 249 bp: TT homozygote, two bands of 135 and 114 bp: CC homozygote, three bands of 249, 135 and 114 bp: CT heterozygote.

The differences in genotype distribution and allele frequencies among groups were evaluated by the Pearson's chi-square and Fisher's exact tests. Haplotype and linkage disequilibrium (LD) were analyzed with Haploview analysis program (18). p values < 0.05 were considered to be statistically significant.

RESULTS

A total of 152 subjects, consisting of 72 patients (62 males and 10 females) and 80 healthy controls were studied. The mean age was 63.9 $\pm$ 11.3 years (min: 36, max: 86 yrs). Disease duration was 8.0 $\pm$ 9.0 years (min: 0, max: 40 yrs). 38 (52.8%) COPD patients were classified according to the stage of the disease as moderate (50% $\leq$ FEV₁ < 80% predicted), 20 (27.8%) as severe (30% $\leq$ FEV₁ < 50% predicted) and 14 (19.4%) as very severe (FEV₁ < 30% predicted). Of the healthy group 41 were cigarette smokers.

We investigated the genotype distribution and allele frequencies of both polymorphisms in the patient and control groups. All genotype distributions of the polymorphisms were consistent with Hardy-Weinberg equilibrium. We found that IL-1 β -511 CT genotype was the most common among groups but distribution of genotypes among groups did not reveal a statistically significant difference (Table 1). We also compared allele frequencies among groups and found that none of the alleles were superior to each other (Table 2). When distribution of genotypes and

Table 1. Distribution of genotypes in patient and control groups

Genotype	Patient group (n= 72)	Healthy smoker group (n= 41)	Healthy non-smoker group (n= 39)	p	Patient vs. healthy smoker OR (95% CI)	Patient vs. healthy non-smoker OR (95% CI)
IL-1 β -511 CC	14 (19.4%)	3 (7.3%)	11 (28.2%)	0.13	-	-
IL-1 β -511 CT	41 (56.9%)	30 (73.2%)	22 (56.4%)		p= 0.06 0.29 (0.08-1.1)	p= 0.43 1.46 (0.57-3.77)
IL-1 β -511 TT	17 (23.6%)	8 (19.5%)	6 (15.4%)		p= 0.3 0.46 (0.1-2.05)	p= 0.2 2.23 (0.66-7.55)
IL-1 β +3954 CC	40 (55.6%)	16 (39%)	18 (46.2%)	0.52	-	-
IL-1 β +3954 CT	30 (41.7%)	23 (56.1%)	19 (48.7%)		p= 0.11 0.52 (0.24-1.16)	p= 0.4 0.71 (0.32-1.58)
IL-1 β +3954 TT	2 (2.8%)	2 (4.9%)	2 (5.1%)		0.58* 0.4 (0.05-3.09)	0.59* 0.45 (0.06-3.45)

* Performed with Fisher's exact test as the cell count was lower than 5.

Table 2. Allele frequencies in patient and control groups

	Patient%	Smoker%	Non-smoker%	Patient vs. healthy smoker	Patient vs. healthy non-smoker
IL-1β-511					
C	69 (47.9)	36 (43.9)	44 (56.4)	p= 0.56 0.85 (0.49-1.47)	p= 0.23 1.41 (0.81-2.45)
T	75 (52.1)	46 (56.1)	34 (43.6)		
IL-1β+3954					
C	110 (76.4)	55 (67.1)	55 (70.5)	p= 0.13 0.63 (0.35-1.15)	p= 0.34 0.74 (0.4-1.37)
T	34 (23.6)	27 (32.9)	23 (29.5)		

Table 3. Distribution of genotypes according to disease stages in patient group

Genotype	COPD stage			p	OR (95% CI)
	Moderate n= 38 (%)	Severe n= 20 (%)	Very severe n= 14 (%)		
IL-1β-511 CC	10 (26.3)	3 (15)	1 (7.1)	0.5	-
IL-1β-511 CT	21 (55.3)	11 (55)	9 (64.2)		p= 0.19 2.38 (0.64-8.84)
IL-1β-511 TT	7 (18.4)	6 (30)	4 (28.6)		p= 0.09 3.57 (0.79-16.2)
IL-1β+3954 CC	20 (52.6)	15 (75)	5 (35.7)	0.12	-
IL-1β+3954CT	17 (44.8)	4 (20)	9 (64.3)		p= 0.58 0.77 (0.30-1.98)
IL-1β+3954 TT	1 (2.6)	1 (5)	-		p= 1.00 1.00 (0.06-17.1)

Severe and very severe groups were merged for statistical calculations.

Table 4. Allele frequencies according to disease staging

	COPD Stage		OR (95% CI)
	Moderate n= 76 (%)	Severe + very severe n= 68 (%)	
IL-1β-511			
C	41 (53.9)	28 (41.2)	0.13 1.67 (0.86-3.24)
T	35 (46.1)	40 (58.8)	
IL-1β+3954			
C	57 (75)	53 (77.9)	0.68 0.85 (0.39-1.84)
T	19 (25)	15 (22.1)	

Severe and very severe groups were merged for statistical calculations.

allele frequencies were investigated according to disease staging, no significant difference was found between these parameters (Table 3,4).

Also, we analyzed the haplotypes of rs16944 and rs1143634 polymorphisms in patient, smoker and non-smoker healthy groups. We found that haplotype frequencies of CT, CC, TC and TT did not differ significantly between patients and control subjects ($p > 0.05$) (Table 5). The linkage disequilibrium of SNPs IL-1β -511 C/T and +3954 C/T was in a strong association ($r^2 = 0.106$, $D' = 0.503$, LOD: 1.82 in patient vs. smoking controls group and $r^2 = 0.142$, $D' = 0.653$, LOD: 2.94 for patient vs. non-smoking control group).

Table 5. Haplotype frequencies of IL-1 β -511 C/T and +3954 C/T polymorphisms in patient and control groups

Haplotype	Haplotype frequency			P	
	Patients	Healthy smoker	Healthy non-smoker	p ^a	p ^b
rs 16944-rs 1143634					
C-T	0.470	0.454	0.405	0.893	0.348
C-C	0.294	0.217	0.300	0.172	0.915
T-C	0.186	0.234	0.264	0.311	0.175
T-T	0.051	0.095	0.031	0.295	0.502

P^a: Patients and healthy smoker.
P^b: Patients and non-healthy smoker.

DISCUSSION

COPD is an enormous cause of global morbidity and mortality that is becoming an even greater health problem with the growing use of tobacco around the world (1). COPD is a complex genetics disease and is caused by the interaction of environmental and genetic factors (6). The major environmental risk factor for the development of COPD is cigarette smoking (1). The other environmental risk factors (lower socio-economics status, low body-mass index, diet etc.) are likely to be much less important than cigarette smoking, but they may interact with smoking to increase the risk of COPD (6). However, only 10-20% of smokers develop clinically significant COPD, suggesting that genetic factors are involved in the pathogenesis. Up-to-date, severe alpha 1-antitrypsin (AAT) deficiency [e.g., protease inhibitor (PI) Z] remains the only proven genetic risk factor for COPD (13,19).

The major risk factor long-term cigarette smoking is associated with activation of a cascade of inflammatory responses (13). It has been proposed that genetic differences in this cascade may play role in the development of COPD. Two pro-inflammatory cytokines, tumor necrosis factor α (TNF- α) and interleukin-1 β (IL-1 β), initiate the innate response and then stimulate the adoptive response (20). As IL-1 β is an important pro-inflammatory cytokine in the inflammatory response, several studies have focused on the effects of this cytokine in various diseases including COPD.

There are several studies investigating the association of polymorphisms of the IL-1 β gene with the development of COPD. However, these studies

report contradictory results. It has been shown that neither of the genotype distributions of polymorphisms IL-1 β -511 C/T or IL-1 β +3954 C/T is associated with the rate of decline in lung function or development of COPD in the Egyptian, Japanese, Caucasian and Indian populations (13-15,21). Contradictory, Lee et al. showed that polymorphisms in IL-1 β -511 C/T and -31 T/C significantly increased the risk of COPD in the Korean population (22).

Two meta-analysis investigating the association of COPD with IL-1 polymorphisms revealed contradictory results. While first meta-analysis showed a decreased risk of susceptibility for COPD suggesting that homozygous individuals (CC and TT) had a decreased risk for COPD when compared with heterozygous individuals (CT), the second meta-analysis showed no significant association between IL-1 β polymorphisms and COPD (23,24). However, a deeper analysis by ethnicity showed that carriers of T allele of IL-1 β -511 and C allele of IL-1 β -31 were at a greater risk for developing COPD in East Asians suggesting that genetic variations in the gene sequence may affect the gene expression rate thus causing susceptibility to COPD (24). It is necessary to widen the investigation to cover different populations other than Asians whether this association depends on the population type. There are only a few studies performing a haplotype analysis. Lee et al. showed that individuals with one or two copies of the IL-1 β CCTC haplotype which were carriers of risk alleles of -3737 C/T, -1464 G/C, -511 C/T and -31 T/C polymorphisms, contribute to the increased risk of developing COPD (22). But in this study IL-1 β +3954 polymorphism was not included in the haplotype analysis. Hegab et al. conducted a haplotype analysis

in Egyptian and Japanese COPD patients and showed that the frequencies of the TC and TT haplotypes of (IL-1 β -31 T/C : IL1 β +3954 C/T) were significantly different between the COPD patients and the controls in Egyptians but not Japanese, suggesting a possible interaction with development of COPD (13).

We conducted a haplotype analysis for -511 C > T and +3954 C > T polymorphisms but as the haplotype frequencies of CT, CC, TC and TT were close to each other, no significant relationship was revealed. These contradictory results might be partly due to ethnic differences under different environmental conditions. IL-1 β is produced and released by stimulation of damage-associated molecular pattern molecules (DAMPs) or pathogen-associated molecular pattern molecules (PAMPs). It is not exactly clear how it is secreted but various release mechanisms exist. Generally speaking, monocytes, macrophages and dendritic cells contribute to the secretion of IL-1 β (25). In various studies IL-1 β expression has been associated with COPD severity and exacerbations. An association between increased IL-1 β expression levels and acute exacerbations, airway neutrophilia and disease severity providing evidence that IL-1 β cytokine is active in COPD pathogenesis has been reported (26). Pauwels et al. have shown that protein levels of IL-1 β were significantly increased in COPD patients with induced sputum showing the role of Nlrp3/caspase-1/IL-1b axis in COPD pathogenesis (27). A more recent study by Fu et al. has reported frequent exacerbations in COPD and asthma patients with higher sputum IL-1 β gene expression (28). There is also evidence that serum IL-1 β levels correlate with the clinical symptoms and severity of COPD (29). The increases in levels may be due to the polymorphisms in control regions. A sequence variation such as single nucleotide polymorphism in gene regulatory regions may alter transcription factor binding thus influencing the gene expression (30). These results confirm the important inflammatory role of IL-1 β in COPD.

By measuring the levels of IL-1 β , asthma and COPD may be distinguished from each other. Damera et al. have shown increased levels of IL-1 β in sputum of acute exacerbated COPD patients (31). Similar to this study, a significant increase in IL-1 β levels was determined in asthmatic patients while there was no significant difference by means of IL-1 β levels between the COPD patients and healthy subjects (17).

Since the susceptibility to COPD is considered to be influenced by multiple genetic causes and genotype-

by-environmental interactions, it is also possible that different polymorphisms in different ethnic groups cause the same COPD phenotype. However, it is still important to confirm the associations of the polymorphisms in different populations (13). Also, it may be plausible to perform a haplotype analysis as a biomarker prediction over a SNP analysis for COPD diagnosis. New candidates need to be assessed in order to improve our understanding of the development of this disease. Recent studies provide substantial evidence for use of IL-1 β as a biomarker of COPD and asthma diagnosis. The limitation of our study is that we did not perform an expression analysis to measure the possible effect of the polymorphism on gene expression rate. With a larger number of patients and controls, including the serum IL-1 β levels may help to develop a diagnostic biomarker.

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