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Evaluation of whether the *ACE* gene I/D polymorphism constitutes a risk factor for chronic obstructive pulmonary disease in the Turkish population

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ABSTRACT. Chronic obstructive pulmonary disease (COPD) is characterized by progressive airflow obstruction that occurs as a result of the normal inflammatory process to protect against harmful irritants and chemicals. Another physiological regulatory process, the renin angiotensin system (RAS), plays an important role in the pathology of many diseases. Angiotensin converting enzyme (ACE) is a key enzyme of RAS. We investigated the frequency of the *ACE* gene I/D polymorphism in patients with COPD in Turkey. This study was performed on 47 unrelated patients with COPD and 64 healthy subjects.

DNA samples were isolated from peripheral blood, and *ACE* DNA was amplified by polymerase chain reaction. The frequencies of *ACE* genotypes were 27.7, 55.3, and 17% for DD, ID, and II in the COPD group, respectively, and 43.8, 43.8, and 12.4% in the control group. There was no statistically significant difference between groups (χ^2 = 3.078; df = 2; P = 0.220). The distributions of *ACE* gene D alleles were 38.2% (N = 52) in the COPD group and 61.8% (N = 84) in the control group; and those of I alleles were 48.8% (N = 42) in the COPD group and 51.2% (N = 44) in the control group. There was no statistically significant difference between groups for allele frequency (χ^2 = 2.419; df = 2; P = 0.120). We believe these results can be useful for large-scale population genetic research considering the frequency of the *ACE* gene variation in COPD patients in the Turkish population.

Key words: Angiotensin converting enzyme; Turkish population; Chronic obstructive pulmonary disease; Renin angiotensin system

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a severe disease that leads to irreversible obstruction of the small airways. The prevalence of COPD is rapidly increasing in developed countries, and it is predicted that this disease will reach the third cause of mortality worldwide (Marin et al., 2011). COPD is characterized by an abnormal excessive inflammatory response of the lung parenchyma to inhaled irritants and toxins, and by the presence of systemic inflammation (Nakawah et al., 2013). Although the most important risk factor for development of COPD is smoking, relationships between genetic and clinical features of COPD have been reported by multiple researchers (Busquets et al., 2007; Lee et al., 2009; Shaw et al., 2012; Simsek et al., 2013). On the other hand, these findings have not been sufficient to clarify the effects of genetic markers on COPD pathogenesis.

The renin angiotensin system (RAS) plays an important physiologic role as a regulator of blood pressure, water homeostasis, cardiovascular remodeling, and vascular tone. Angiotensin converting enzyme (ACE) is a key component of RAS. The *ACE* insertion/deletion (I/D) gene polymorphism, which comprises a 287-bp fragment placed within the intron 16 region of the *ACE* gene, has been identified in different clinical cases such as acute myocardial infarction, cardiac hypertrophy, and failure (Schuster et al., 1995; Holmer and Schunkert, 1996). Presence of the D allele has been associated with increased circulating levels of the ACE enzyme; it has been shown that individuals carrying the DD, ID, and II genotypes have the highest, intermediate, and lowest circulating levels of ACE, respectively. The *ACE* I/D polymorphism has been found to exhibit differences in various ethnic and patient populations (Rigat et al., 1990; Turgut et al., 2004; Sabbagh et al., 2007; Li et al., 2013). It is believed that inhibition of ACE activity can, in the long term, provide benefits for treatment of patients with COPD through its effects on inflammation, respiratory function, and peripheral oxygen delivery (Forth and Montgomery, 2003).

If we consider *ACE* I/D polymorphic variation across populations to have benefit for therapeutic response or expression of COPD, it is necessary to know more about this polymorphism in different populations. In this study, we aimed to identify genotype and allele frequencies of *ACE* I/D polymorphisms in patients with COPD in the Kütahya Province of Turkey.

MATERIAL AND METHODS

Participants

We performed this study utilizing a total of 111 subjects who were treated at the Department of Thoracic Medicine, Dumlupınar University, Kütahya, Turkey. Forty-seven unrelated patients with COPD were included as the patient group and 64 healthy age-matched subjects were included as the control group. Both groups were chosen from among the Turkish population. The diagnosis of COPD was established on the basis of criteria proposed by the Global Initiative for Chronic Obstructive Lung Disease (GOLD Guideline, 2014). All procedures were explained individually to all subjects and written informed consent was obtained. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki as reflected in a prior approval by the institution's human research committee. The study was approved by the Clinical Research Ethics Committee of Afyon Kocatepe University.

Detection of *ACE* gene polymorphisms

DNA isolation

Blood samples were collected in tubes with ethylenediaminetetraacetic acid from all subjects. A total of 111 genomic DNA samples were isolated from peripheral blood leukocytes by a standard phenol/chloroform extraction method as previously described in detail (Turgut et al., 2004).

Polymerase chain reaction (PCR)

PCR was used to detect the I and D alleles in intron 16 of the *ACE* gene by using upstream 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' and downstream 5'-GAT GTG GCC ATC ACA TTC GTC AGAT-3' primers. PCR was conducted in 50-µL reaction mixtures containing approximately 1 µg DNA sample, 5 µL 10X reaction buffer incomplete [160 mM $NH_4{}_2SO_4$, 670 mM Tris-HCL, pH 8.8, 0.1% Tween-20], 5 µL 2 mM dNTPs, 3 µL 25 mM $MgCl_2$, 1 U Taq-polymerase, and 100 pmol of each primer. Amplification was performed for 35 cycles, each cycle including denaturation, extension, and annealing temperatures of 94°C for 30 s, 60°C for 15 s, and 72°C for 30 s, respectively, and a final extension at 72°C for 4 min. An initial denaturation stage was carried out at 95°C for 2 min. The amplified fragments were separated by electrophoresis on a 2% agarose gel and visualized by ethidium bromide staining under ultraviolet light. The polymorphism was detected as a 490-bp fragment in the presence of the insertion (I allele) and as a 190-bp fragment in the presence of the deletion (D allele). Each sample was described as DD, ID, or II.

Statistical analysis

Statistical analyses were performed using the SPSS 16.0 software (SPSS; Chicago, IL, USA). All data are reported as means ± standard deviation. The chi-square test was used for comparison of nominal variables between groups. Statistical significance of the observed genotype frequencies was evaluated according to the Hardy-Weinberg rule and compared

to the expected genotype frequencies. Hardy-Weinberg equilibrium was evaluated by the chi-square test (Rodriguez et al., 2009). All P values < 0.05 were accepted as statistically significant.

RESULTS

The frequency of *ACE* I/D polymorphism genotypes in controls and patients did not show a significant deviation from Hardy-Weinberg equilibrium (P > 0.05) (Table 1). The frequencies of *ACE* genotypes in patients with COPD and in control subjects are shown in Table 2 and Figure 1. The distribution of *ACE* genotypes were found to be 27.7% (13) for DD, 55.3% (26) for ID, and 17% (8) for II in the COPD group and 43.8% (28), 43.8% (28), and 12.4% (8) for DD, ID, and II, respectively, in the control group. There was no statistically significant difference between groups for *ACE* genotype frequencies ($\chi^2 = 3.078$; df = 2; P = 0.220).

Table 1. Hardy-Weinberg equilibrium of the *ACE* gene I/D polymorphism.

		COPD		Control	
	Genotype	Expected	Observed	Expected	Observed
Common homozygotes	DD	14.38	13	27.56	28
Heterozygotes	ID	23.23	26	28.88	28
Rare homozygotes	II	9.38	8	7.56	8
		$\chi^2 = 0.67$; P > 0.05		$\chi^2 = 0.06$, P > 0.05	

COPD = chronic obstructive pulmonary disease.

Table 2. Genotype and allele frequencies of the *ACE* gene I/D polymorphism.

	COPD		Control	
	N	%	N	%
Genotype Frequency				
ACE I/D polymorphism				
DD	13	27.7	28	43.8
ID	26	55.3	28	43.8
II	8	17.0	8	12.4
Total	47		64	
$\chi^2 = 3.078$; df = 2; P = 0.220				
Allele Frequency				
ACE D allele	52	38.2	84	61.8
ACE I allele	42	48.8	44	51.2
$\chi^2 = 2.419$; df = 2; P = 0.120				

COPD = chronic obstructive pulmonary disease; df = degrees of freedom.

The allele frequencies for the *ACE* gene in patients with COPD and in control subjects are shown in Table 2. The distributions of *ACE* D alleles were found to be 38.2% (52) in the COPD group and 61.8% (84) in the control group; I alleles were found at 48.8% (42) and 51.2% (44) in the COPD and control groups, respectively. There was no statistically significant difference between groups for allele frequency ($\chi^2 = 2.419$; df = 2; P = 0.120) (Table 2, Figure 2).

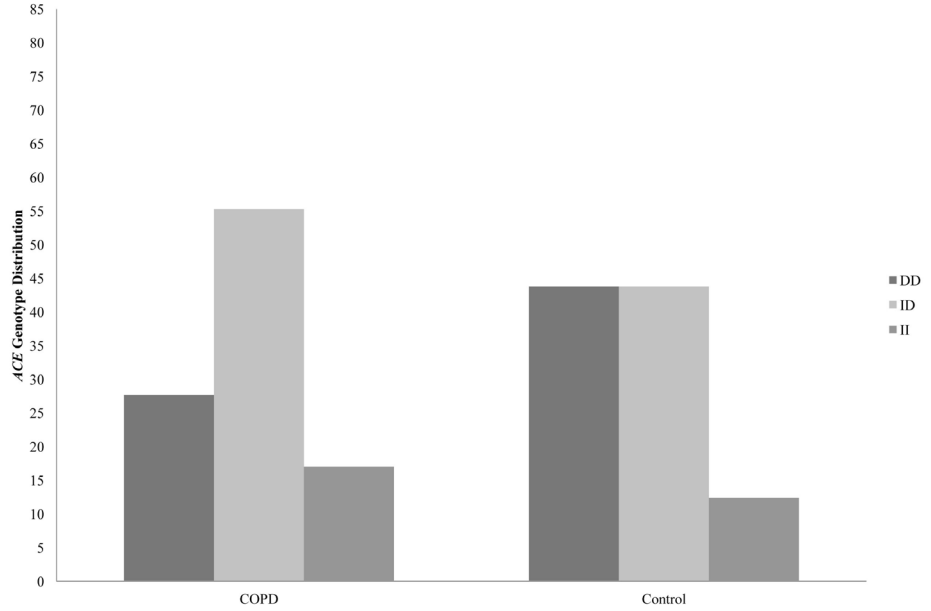

Figure 1. *ACE* genotype distributions in patients with COPD and control subjects (%). *ACE*, angiotensin converting enzyme gene; COPD, chronic obstructive pulmonary disease.

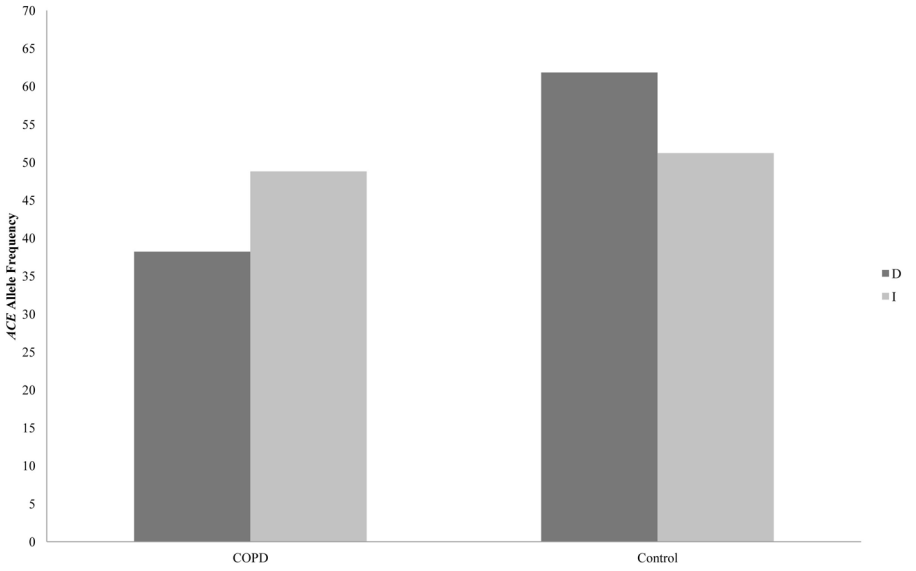

Figure 2. Frequency of *ACE* D and I alleles in patients with COPD and control subjects. *ACE*, angiotensin converting enzyme gene; COPD, chronic obstructive pulmonary disease.

DISCUSSION

COPD is a highly prevalent chronic disease in the general population and is characterized by heterogeneous chronic airway inflammation and airway obstruction. Chronic inflammation affects the whole respiratory tract, from the central to peripheral airways, via different inflammatory cells and mediators. Thus, responses to therapy differ between patients with COPD. Airway obstruction in COPD occurs progressively and irreversibly (Marin et al., 2011; Nakawah et al., 2013). ACE in mononuclear cells may participate in the local production or degradation of regulatory peptides of inflammation reactions. Some inflammatory peptides such as bradykinin and substance P are partially inactivated by ACE. It is known that RAS endocrine activities are associated with the regulation of vascular tonus and cardiac function in the body. On the other hand, RAS autocrine activities also contribute to inflammation reactions in tissues (Hollá et al., 1999). ACE is attractive as a candidate to play a role in the development of vascular pathological states (Kennon et al., 1999).

ACE polymorphic variation has been identified in association with different clinical features of COPD, such as a disturbance in peripheral tissue oxygenation during exercise (Kanazawa et al., 2002), the regulation of skeletal muscle aerobic work efficiency (Zhang et al., 2008), the severity of skeletal muscle weakness (Hopkinson et al., 2004), and pulmonary hypertension evoked by exercise challenge (Kanazawa et al., 2000). Several molecular epidemiological studies have been conducted to evaluate the risk of COPD association with the I/D polymorphism of the *ACE* gene. However, the results have been conflicting (Li et al., 2013). Busquets et al. (2007) have identified an association of *ACE* I/D genotype with smoking history and risk for developing COPD. On the other hand, Lee et al. (2009) reported no such association.

Recently, Simsek et al. (2013) reported a significant difference in the I/D allele frequencies of the *ACE* gene between COPD and control groups; their study was performed in a Turkish population living in the eastern geographical region of Turkey. By contrast, in our study, we could not observe any statistically significant difference in I/D allele frequencies or genotypes of the *ACE* gene between COPD and control groups. Our study was performed in the Turkish population of the Kütahya Province of the Aegean geographical region of Turkey, which is the western part of Turkey. We know from meta-analysis that the D allele and the DD homozygous genotype might be significant molecular genetic markers for COPD susceptibility in Asians, but not Europeans (Li et al., 2013). This geographic phenomenon might be the explanation for the differences found between the previous study and our research. We note, however, that in the COPD group, although not statistically significant, the DD genotype and D allele frequencies were lower than those in the control group. If we consider that a higher plasma level of ACE is dependent on the DD genotype, for this population base, we can suggest that the I allele might have a protective role against COPD development. In the COPD group, the D allele was less frequent than the I allele but not significantly. This might also show that the I allele might have a protective role against the development of comorbidity in COPD patients, which is dependent on higher plasma levels of ACE.

In conclusion, the results of the present study demonstrated that patients with COPD in the Kütahya Province of Turkey do not differ from healthy subjects for *ACE* gene I/D polymorphism. Our results can serve as a basis for large-scale studies on the relationship of the I/D genotype in COPD in the Turkish population. We also believe that investigation of genotype and allele frequencies related to RAS substrates such as angiotensinogen, ACE2, and angiotensin receptors, which might be related to COPD pathogenesis, should be investigated at a re-

gional basis in the Turkish population. Data obtained from such studies could help to understand the development of COPD and its relation to population genetics, in consideration with variations in genotype frequency possibly occurring throughout the history of the Anatolian basin.

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