

# Lack of association of ACE gene I/D polymorphism with obstructive sleep apnea syndrome in Turkish patients

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**ABSTRACT.** Angiotensin-converting enzyme (ACE) is a vital enzyme in the renin-angiotensin-aldosterone system, and there are reports in the literature describing its role in the development of cardiovascular system diseases, with I/D polymorphism of the ACE gene. We examined the relationship between a patient group with obstructive sleep apnea syndrome (OSAS) and a control group in terms of I/D polymorphism of the ACE gene. We examined 64 patients, with 37 individuals serving as the control group. PCR was used to detect ACE I/D gene polymorphism. Genotype was determined according to the bands that formed on agarose gel electrophoresis. Among the 64 OSAS patients, 27 were identified with the ID genotype, 27 with the DD genotype and 10 with the II genotype; among the 37 control subjects, 19 were identified with the ID genotype, 11 with the DD genotype and 7 with the II genotype. When the case group and controls were compared in terms of ID, II and DD genotypes, no significant difference was observed. On the other hand, when the two groups were compared with respect to mean body

mass index, the OSAS group was found to be significantly different from the control group ( $P = 0.009$ ). We conclude that ACE I/D gene polymorphism is not a genetic risk factor for OSAS in Turkish patients.

**Key words:** Polymorphism; ACE gene; Obstructive sleep apnea syndrome

## INTRODUCTION

Obstructive sleep apnea syndrome (OSAS) is characterized by repetitive episodes of upper respiratory tract obstruction during sleep and frequent decrease in arterial oxygen saturation, and its prevalence in a community is determined to be 3.9% among men and 1.2% among women at ages of 20 to 100 years. It is reported to be a disease that is more prevalent than asthma and diabetes mellitus among adults (Ursava and Ege, 2003; Yavuz et al., 2008; Ahmadi et al., 2009). Among patients with OSAS, there is an increased incidence of various cardiovascular diseases including hypertension, stroke, acute myocardial infarct, and arrhythmia complications, and it has been shown to be related to cardiovascular mortality-morbidity rate (Barceló et al., 2001; Yaggi et al., 2005).

Angiotensin-converting enzyme (ACE) is a vital enzyme in the renin-angiotensin-aldosterone system, and is reported to play a role in the development of cardiovascular system diseases with I/D polymorphism of the ACE gene (Butler et al., 1997; Candy et al., 1999; Agerholm-Larsen et al., 2000). There are reports that individuals with the D allele and especially the DD genotype are prone to hypertension (O'Donnell et al., 1998; Bengtsson et al., 1999).

To date, there are no published studies of the ACE I/D gene polymorphism in relation to OSAS risk in Turkish patients with OSAS. The aim of this study was to determine the relationship between a group of patients with OSAS ( $AHI \geq 5$ ) and a control group ( $AHI < 5$ ) in terms of I/D polymorphism of the ACE gene.

## MATERIAL AND METHODS

### Patients

This study involved 101 individuals who were classified based on the apnea-hypopnea index (AHI) determined by a standard polysomnography conducted by the Thoracic Diseases Department of the School of Medicine at Uludag University. The sum of apnea and hypopnea episodes was divided by the total sleep time to obtain the AHI score. Patients with  $AHI \geq 5$  were considered to have OSAS. Subjects with  $AHI < 5$  were included in the control group. Among these cases, 64 formed the OSAS group while 37 cases formed the control group. Demographic characteristics (age, gender) and body mass index (BMI) were noted for all individuals. The study was approved by the local Ethics Committee.

### Methods

Blood samples were obtained in EDTA tubes both from the patients and controls. DNA was isolated according to the Dr. Zeydanlı DNA isolation kit procedure and then stored at  $-20^{\circ}\text{C}$  until polymerase chain reaction (PCR) was performed. The ACE I/D gene polymor-

phism from DNA samples was determined using the PCR technique.

The primers used to determine the ACE I/D polymorphism were F: 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' and R: 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3', and the DD genotype insertion area specific primer: F: 5'-TGG GAC CAC AGC GCC CGC CCG CCA CTA C-3' and R: 5'-TCG CCA GCC CTC CCA TGC CCA TAA-3' (Lee and Tsai, 2002). A PCR mixture of 30  $\mu$ L was prepared for the ACE gene amplification from DNA samples. The mixture was prepared to contain 2.5  $\mu$ L 10X Taq polymerase buffer, 0.5  $\mu$ L 10 mM dNTP mixture, 2  $\mu$ L 25 mM MgCl<sub>2</sub>, 1  $\mu$ L 10 pmol primary couple, 0.2  $\mu$ L Taq polymerase (Bioron) and 20  $\mu$ L ddH<sub>2</sub>O for each sample. About 3  $\mu$ L (100 ng) DNA sample was added to the mixture. To prevent wrong DD genotyping in samples of the ACE DD genotype, the results were confirmed with a second PCR analysis. PCR conditions were as follows: a first denaturation for 5 min at 94°C, followed by a second denaturation for 1 min at 94°C, which continued with 35 cycles that consisted of annealing for 1 min at 57°C (for verification of the DD genotype, at 63°C), and extension for 1 min at 72°C, which ended with final extension for 10 min at 72°C.

After PCR, the samples were separated by 2% agarose gel electrophoresis, stained with ethidium bromide and photographed for the amplification study. As a result of the amplification study, the agarose gel showed an amplification band of 190 bp in samples with the DD genotype, bands of 490 and 190 bp in samples with the ID genotype and a band of 490 bp in samples with the II genotype. In the second PCR analysis conducted for DD confirmation, an amplification band of 335 bp was observed with samples that had the insertion band.

## Statistical analysis

All data are reported as means  $\pm$  standard deviations. The SPSS 13.0 program was used for the analysis. The Mann-Whitney test was used for comparisons made between the groups in terms of BMI and age, while the chi-square test was used for comparison of ACE gene polymorphism. A P value of <0.05 was accepted to be statistically significant.

## RESULTS

In this study, among the 64 cases in the patient group (53 males and 11 females), the average age was  $50.37 \pm 11.2$  years, and among the 37 cases in the control group (26 males and 11 females), the average age was  $49.97 \pm 10.4$  years. The average BMI was found to be significantly different between the patient group with OSAS ( $30.64 \pm 4.3$  kg/m<sup>2</sup>) and the control group ( $28.51 \pm 4.6$  kg/m<sup>2</sup>) (P = 0.009) (Table 1).

**Table 1.** Clinical characteristics of obstructive sleep apnea syndrome (OSAS) patients and the control group.

	OSAS patients (AHI $\geq$ 5) (N = 64)	Control group (AHI < 5) (N = 37)	P
Gender (male/female)	53/11	26/11	P > 0.05
BMI (kg/m <sup>2</sup> )	$30.64 \pm 4.3$	$28.51 \pm 4.6$	P = 0.009
Age (years)	$50.37 \pm 11.2$	$49.97 \pm 10.4$	P > 0.05

AHI = apnea-hypopnea index; BMI = body mass index.

No significant differences in allele and genotype frequencies for any polymorphism were observed between patients and controls, although the D allele was more frequent in patients (P > 0.05) (Table 2).

**Table 2.** Allele frequency and genotype distribution among obstructive sleep apnea syndrome (OSAS) patients and the control group.

	OSAS patients (AHI > 5) (N = 64)	Control group (AHI < 5) (N = 37)	OR (95%CI)	P
II genotype	10	7	0.79 (0.24-2.61)	P > 0.05
ID genotype	27	19	0.69 (0.28-1.69)	P > 0.05
DD genotype	27	11	1.72 (0.67-4.48)	P > 0.05
I allele (%)	47 (37%)	33 (45%)	0.79 (0.43-1.46)	P > 0.05
D allele (%)	81 (63%)	41 (55%)		

Regarding the I/D polymorphism of the ACE gene in the study, among the 64 OSAS patients, 27 were identified with the ID genotype, 27 with the DD genotype, and 10 with the II genotype. Among the 37 individuals in the control group, 19 were identified with the ID genotype, 11 with the DD genotype and 7 with the II genotype. In the patient group, the frequency of the I allele was 37% and the frequency of the D allele was 63%, and in the control group the frequency of the I allele was determined to be 45% and the frequency of the D allele was 55%.

## DISCUSSION

In the 1990's, the renin-angiotensin-aldosterone system, especially angiotensin II, was shown to play an important role in the pathogenesis of cardiovascular and kidney diseases. ACE catalyzes the conversion of angiotensin I to angiotensin II, and its serum activity has been shown to account for some cardiovascular diseases. It has been reported that the I/D polymorphism of the ACE gene (Nakai et al., 1994; Schunkert et al., 1994) contributing to ACE serum activity may have a relationship with the development of cardiovascular diseases (Raynolds et al., 1993; Seekin et al., 2006).

In our study, we investigated the relationship between the ACE polymorphism with a group of Turkish OSAS patients and a control group. No significant difference was found between the OSAS group and the control group in terms of genotype and allele frequency ( $P > 0.05$ ). Similarly, in an ACE I/D polymorphism analysis among the OSAS patients, it was observed that there is no difference between patient and control groups in terms of I/D polymorphism distribution (Barceló et al., 2001; Rubinsztajn et al., 2004; Patel et al., 2007). In our literature search, we found that only Xiao et al. (1999) showed that the frequency of the I allele is significantly higher in OSAS patients. Contrary to the study of Xiao et al., we found no significant difference in allele frequency of the ACE I/D polymorphism among OSAS patients (Barceló et al., 2001; Rubinsztajn et al., 2004; Patel et al., 2007).

On the other hand, when we compared the groups in terms of BMI, a statistically significant difference was determined between patient and control groups ( $P = 0.009$ ). The BMI of the OSAS group was higher when compared with the individuals in the control group. The BMI results for OSAS patients showed similarity with those of other polymorphism studies (Barceló et al., 2001; Rubinsztajn et al., 2004; Sakai et al., 2005; Piérola et al., 2007).

Further studies of patients in larger numbers and of different ethnic backgrounds may be necessary to elucidate the association between the ACE I/D gene polymorphism and increased risk of OSAS.

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