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Insertion/Deletion Polymorphism and Serum Activity of the Angiotensin-Converting Enzyme in Turkish Patients with Obstructive Sleep Apnea Syndrome

Candan Ogus · Serap Ket · Turker Bilgen · Ibrahim Keser · Aykut Cilli · Ayse Yesim Gocmen · Ozgur Tosun · Saadet Gumuslu

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Abstract This study determined the allelic frequency and genotypic distribution of an angiotensin-converting enzyme (ACE) polymorphism and serum ACE activity in Turkish patients with obstructive sleep apnea syndrome (OSAS). A colorimetric assay measured serum ACE activity in 73 of 97 subjects. Frequencies for II, ID, and DD genotypes were 19.6, 53.6, and 26.8% in the OSAS group and 15, 38, and 47% in the control group, respectively ($P = 0.02$). The I allele frequency was higher in the OSAS group than in the healthy control group ($P = 0.02$). Carrying the I allele (II or ID genotypes) increased OSAS risk 2.41 times in the Turkish population. Mean ACE activity was significantly lower in patients with the II genotype than in the DD genotype ($P = 0.011$), and ACE activity was significantly lower in patients with severe OSAS than in those with mild OSAS ($P = 0.006$). Our results suggest that II and ID genotypes of the *ACE* gene increase the risk of developing OSAS in the Turkish population.

C. Ogus (✉) · A. Cilli
Department of Chest Diseases, School of Medicine, Akdeniz University, 07058 Antalya, Turkey
e-mail: candanogus@akdeniz.edu.tr

S. Ket
Department of Chest Diseases, Doruk Medical and Surgical Center, Bursa, Turkey

T. Bilgen · I. Keser
Department of Medical Genetics, School of Medicine, Akdeniz University, 07058 Antalya, Turkey

A. Y. Gocmen · S. Gumuslu
Department of Biochemistry, School of Medicine, Akdeniz University, 07058 Antalya, Turkey

O. Tosun
Department of Biostatistics and Medical Informatics, School of Medicine, Akdeniz University, 07058 Antalya, Turkey

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Introduction

The modern obesity pandemic is likely to increase the prevalence of obstructive sleep apnea syndrome (OSAS), the most common form of sleep-disordered breathing (Taheri 2004; Taheri and Mignot 2002). OSAS is associated with snoring, apnea, daytime sleepiness, and significant mortality due to accidents and cardiovascular events (Ursavas et al. 2007). Therefore, understanding the pathophysiologic basis of OSAS is essential for the development of prevention, screening, and therapeutic strategies.

First-degree relatives of patients with OSAS have been shown to be at high risk for development of this disorder. Familial aggregation studies indicate that most of the OSAS risk factors were obesity, ventilatory control abnormalities, and craniofacial dysmorphism (Taheri and Mignot 2002; Kaparianos et al. 2006). The study of Palmer et al. suggests the involvement of multiple genetic factors associated with development of OSAS (Palmer et al. 2004). Although several genes may increase the risk of OSAS, the molecular basis of OSAS development has not been clearly elucidated (Taheri 2004; Riha et al. 2005; Tafti et al. 2007; Bayazit et al. 2006a, b, 2007; Hanaoka et al. 2008; Pierola et al. 2007; Barcelo et al. 2002).

Circulating angiotensin-converting enzyme (ACE) activity shows extensive interindividual variability, and *ACE* insertion (I)/deletion (D) polymorphism accounts for 47% of the total variance of serum ACE levels (Rigat et al. 1990). Plasma and tissue levels of ACE activity are higher in patients with the DD genotype than in those with the II genotype, and patients with the ID genotype have intermediate ACE levels (Seckin et al. 2006; Ozen et al. 1997). Experimental and anthropological studies indicate that I polymorphism in the *ACE* gene, which produces reduced serum and tissue ACE activity, is more frequent in individuals with greater endurance and better adaptation to high altitude (Palmer and Redline 2003). Of the few studies investigating the relationship between ACE I/D polymorphism and OSAS, Barcelo et al. (2001) did not find any difference in frequency distribution of the DD, II, and ID genotypes between OSAS patients and healthy subjects. Rubinsztajn et al. (2004) also reported no association between ACE polymorphisms and OSAS. Xiao et al. (1999), however, described the I allele as a risk factor for OSAS in a Chinese population. In addition, a high frequency of the I allele and the II genotype has been closely associated with hypertensive patients who show more severe forms of OSAS (Zhang et al. 2000). In our study, we determined the allelic frequency, genotypic distribution, and serum levels of ACE activity in Turkish patients who presented with OSAS.

Materials and Methods

The study included 97 unrelated Turkish patients (nine women, 88 men) with OSAS, who were diagnosed with polysomnography between 2001 and 2004. The

study was conducted at the Sleep Unit of the Department of Chest Diseases of the Akdeniz University Medical Faculty. Polysomnography was performed with 16-channel EMBLA SX Proxy 3.0 (Medcare, Iceland) with continuous sleep-technician monitoring, consisting of four channels of EEG, two channels of EOG, submental EMG, oronasal air flow, thoracic and abdominal movements, pulse oximeter saturation, tibial EMG, body position detector, electrocardiogram, and tracheal sound. Records were scored at intervals of 30 s. Apnea was defined as complete cessation of airflow lasting $\geq$10 s. Hypopnea was defined as 70% or more reduction in respiratory airflow lasting $\geq$10 s and accompanied by a decrease of $\geq$4% in oxygen saturation. An apnea–hypopnea index (AHI; average number of episodes of apnea and hypopnea per hour of sleep) was used to determine the degree of OSAS. An index of 5–15 was considered mild, 15–30 moderate, and more than 30 severe OSAS. Sleep data were staged according to the system described by Rechtschaffen and Kales (1968).

All the subjects underwent a physical examination, including body mass index (kg/m^2), sex, AHI, age, neck circumference, and sleep parameters. We evaluated the healthy control groups in the study of Berdeli and Cam (2009), and 79 age-matched healthy Turkish volunteers (mean age 60.1 $\pm$ 10 years) without OSAS from the study group of Tuncer et al. (2006) were adopted as a control group for our study. Patients with sarcoidosis, chronic obstructive pulmonary disease, diabetes mellitus, liver cirrhosis, thyroid dysfunction, or renal failure were excluded. Patients who used ACE inhibitors, AT receptor blockers, and continuous positive airway pressure were also excluded from the study. All participants signed an informed consent form, and this study was approved by the local ethics committee of the Medical Faculty of Akdeniz University.

Genotyping for ACE I/D Polymorphism

Genomic DNA was extracted from 10 ml peripheral blood samples with K_3-EDTA by a salting-out method (Miller et al. 1988). The D and I alleles were identified by polymerase chain reaction (PCR) performed in a final volume of 50 µl containing 10 pmol of each primer (Forward 5′-CTGGAGACCACTCCCATCCTTTCT-3′ and Reverse 5′-GATGTGGCCATCACATTCGTCAGAT-3′), 20 mM dNTP, 1.5 mM $MgCl_2$, 0.5 µg DNA, 5 µl 10 × PCR buffer, and 1 U *Taq* DNA polymerase. The thermal cycling procedure consisted of initial denaturation at 95°C for 5 min, denaturation at 94°C for 1 min, annealing at 63°C for 1 min, and extension at 72°C for 2 min, repeated for 35 cycles (Jeng et al. 1998). The DNA products were visualized in 2% agarose gel stained with ethidium bromide. A fragment of 190 bp represented the D allele, and a fragment of 490 bp represented the presence of the I allele. To prevent mistyping of ID genotypes as DD genotypes, because of the selective amplification of the short fragment, each sample that had the DD genotype was reamplified with insertion-specific primers (Forward 5′-TGGGACCAC AGCGCCCGCCACTAC-3′ and Reverse 5′-TCGCCAGCCCTCCCATGCCCA TAA-3′), which recognizes the inserted DNA sequence in 25 ml of the reaction mixture, with 1 min at 94°C, followed by 30 cycles of 30 s at 94°C, 45 s at 67°C,

and 2 min at 72°C. The 335-bp product was observed in the presence of the I allele (Shanmugam et al. 1993).

Serum ACE Activity

Blood samples were collected, and serum was prepared, and stored at −80°C until analysis. Serum ACE activity was determined with an ACE colorimetric assay kit (KK-ACE, Bühlmann Laboratories AG, Switzerland). One unit of ACE activity was defined as the amount of enzyme required to release 1 μmol of hippuric acid/min/liter of serum at 37°C.

Statistical Analysis

The distribution of I/D polymorphisms of the *ACE* gene in OSAS patients and in the control groups was compared by a chi-square test. The association between the distribution of I and D alleles and OSAS severity subgroups, as well as case and control groups, was assessed by a chi-square test. The ACE plasma activity for three OSAS severity subgroups and three genotypic subgroups was assessed by Kruskal–Wallis variance analysis. A Mann–Whitney *U*-test was used to determine the multiple comparisons in these subgroups. Pearson correlation analysis was used to determine the possible relationship between the study variables and ACE plasma activity. All the statistical analyses were carried out with MedCalc software (version 10.2.0.0). *P*-values lower than 0.05 were considered statistically significant.

Results

Angiotensin-converting enzyme (ACE) genotype distribution was consistent with Hardy–Weinberg equilibrium in both the patient and control groups (Table 1). The I allele was observed more frequently in OSAS patients than in the control group [$P = 0.02$; OR = 1.68 (1.08–2.57)]. Carrying the I allele (genotype II or ID) increases the OSAS risk 2.41 times in the Turkish population [$P = 0.006$; OR = 2.41 (91.28–4.52)].

Table 1 Frequency of *ACE* I/D genotypes and alleles in OSAS patients and control subjects without OSAS

Group (*n*)	Genotype %*			Allele*	
	II	ID	DD	I	D
OSAS patients (97)	19.6	53.6**	26.8**	0.46	0.54
Control subjects (79)	15	38	47	0.34	0.66

* Difference between groups significant at $P < 0.05$. Allele frequency, OR = 1.68 (1.08–2.57)

** Significant at $P < 0.01$. Difference between ID or DD genotype, OR = 2.47 (1.26–4.84). Difference between carrying I allele or not, OR = 2.41 (1.28–4.52)

There were no significant differences in the mean value of neck circumference, body mass index, age, sex, mean duration apnea, and minimum SpO_2 between the I and D genotypes of OSAS patients ($P > 0.05$). Also, we found no statistically significant differences related to degree of severity of OSAS and *ACE* gene polymorphism (Table 2, $P = 0.831$).

Although ACE genotype distribution was determined in all of the patients, serum ACE activity was determined in only 73 patients. We found a statistically significant difference among the ACE genotype subgroups in terms of the mean ACE activity ($P = 0.043$), and the mean ACE activity was lower in the II genotype than in the DD genotype (Table 3, $P = 0.011$). When we compared ACE activity with the severity of OSAS ($P = 0.019$), the ACE activity was significantly lower in the severe OSAS group than in the mild OSAS group (Table 4, $P = 0.006$).

Our investigation of possible relationships between ACE activity and demographic and/or polysomnography variables found significant correlations for both minimum SpO_2 and AHI (Table 5). The level of minimum SpO_2 was high and AHI tended to be low for patients who had higher ACE activity (minimum SpO_2: $r = 0.30$, $P = 0.01$; AHI: $r = -0.31$, $P = 0.007$).

Discussion

Our study showed that the genotypic distribution of *ACE* gene polymorphism was significantly different in the OSAS patients, and the *ACE* II genotype increased

Table 2 Relationship between *ACE* gene polymorphism and OSAS severity

OSAS (*n*)	Genotype *n* (%)		
	II	ID	DD
Mild (44)	10 (22.7)	21 (47.7)	13 (29.5)
Moderate (22)	3 (13.6)	13 (59.1)	6 (27.3)
Severe (31)	6 (19.4)	18 (58.1)	7 (22.6)

Table 3 ACE activity level in three *ACE* genotypes of OSAS patients

* Difference between II and DD genotypes significant at $P < 0.05$

Genotype	ACE activity, mean IU/l ± SD
II	33.53 ± 9.31*
ID	38.25 ± 10.80
DD	41.81 ± 9.62

Table 4 Relationship between mean ACE activity and OSAS severity

* Difference between mild and severe OSAS patients significant at $P < 0.05$

OSAS (*n*)	ACE activity, mean IU/l ± SD
Mild (36)	41.41 ± 10.16*
Moderate (18)	36.96 ± 10.44
Severe (19)	33.31 ± 9.34

Table 5 Correlation analysis of ACE plasma activity and patient variables

Variable	OSAS study population[a] Mean ± SD	ACE plasma activity, *r* (*P*)
Age in years	51.27 ± 9.97	0.80 (0.50)
Body mass index in kg/m^2	30.58 ± 5.79	−0.03 (0.80)
Neck circumference in cm	41.82 ± 3.31	−0.17 (0.16)
Apnea-hypopnea index	24.24 ± 18.34	−0.31 (0.01)*
Minimum SpO_2 percentage	76.52 ± 11.40	0.30 (0.01)*
Epworth score	11.34 ± 4.7	−0.11 (0.35)
Mean duration apnea in seconds	21.55 ± 5.89	0.02 (0.88)

[a] Of the 97 unrelated Turkish patients, 9 (9.3%) were women, 88 (90.7%) were men

* Statistically significant correlation at 0.05 level

OSAS risk 2.4 times in the Turkish population (Table 1). Even though the II genotype was shown to be a high risk factor for OSAS in a Chinese population, no association was found between *ACE* polymorphism and OSAS in Spanish and Polish populations (Barcelo et al. 2001; Rubinsztajn et al. 2004; Xiao et al. 1999).

Barley et al. (1994) studied *ACE* gene polymorphism in different populations, including white Europeans, black Nigerians, Samoan Polynesians, and Yanomami Indians. They found that I allele frequency was higher in the latter two populations, and they concluded that *ACE* gene polymorphism was associated with ethnic origin. In the control group adopted for our study, the frequency of the I allele was found to be 0.34 (Tuncer et al. 2006).

A correlation between homozygote gene deletion (DD genotype) and high ACE activity has been reported in many studies (Seckin et al. 2006; Ozen et al. 1997). Accordingly, we found the highest ACE activity in the DD genotype and the lowest in the II genotype (Table 3). Barcelo et al. (2001) compared ACE activity in patients with OSAS and control subjects and showed that ACE activity is increased in patients with OSAS regardless of the presence or absence of arterial hypertension. We did not evaluate ACE activity in control subjects, which might be a limitation of our study. When we compared ACE activity with the severity of OSAS, the ACE activity was significantly lower in the severe OSAS group than in the mild OSAS group, although genotype differences do not exist between the two groups (Table 4). We found that the level of minimum SpO_2 was significantly higher and AHI was significantly lower in patients with high ACE activity (Table 5).

Several possible mechanisms may contribute to our results. First, different *ACE* genotype distributions can result in different ACE activity levels. We found no significant correlations, however, between OSAS severity and *ACE* gene polymorphism. Second, morbid obesity can negatively influence ACE activity. Previous results have shown the involvement of ACE in adipocyte growth, function, and inhibition of adipocyte differentiation by ACE-processed angiotensin II, but Bell et al. (2007) reported no correlation between ACE gene variations and development of severe obesity. To our knowledge, no study has defined ACE serum activity in

obese individuals. Third, decreased serum activity of ACE depends on intermittent hypoxemia related to high frequency apnea/hypopnea episodes in severe OSAS. In our study, we found that SpO_2 was higher and AHI was lower in patients with high ACE activity (Table 5). The ACE enzyme activity can change in pulmonary diseases due to vascular endothelial damage. Mean serum ACE activity has been reported for a wide variety of chronic airway diseases such as asthma, chronic bronchitis, emphysema, and cystic fibrosis. Rohatgi (1982) reported lower ACE activity in OSAS patients than in healthy controls. Ashutosh and Keighley (1976) and Kanazawa et al. (2000), however, reported higher ACE activity in chronic hypoxia. Further studies are needed to clarify these discrepancies.

In conclusion, our data demonstrate that the frequency of the II genotype of the *ACE* gene is significantly higher in OSAS patients than in healthy subjects, and the II genotype increases the risk of development of OSAS by 2.4 fold in this Turkish population.

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