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Roles of beta2-adrenergic receptor gene polymorphisms in a Turkish population with obstructive sleep apnea syndrome or obesity

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ABSTRACT. We determined the distribution of the Arg16Gly and Gln27Glu polymorphisms of the beta-2 adrenergic receptor gene (*ADRB2*) in patients with obstructive sleep apnea syndrome as well as a control group in Northeastern Turkey. A total of 52 patients diagnosed with obstructive sleep apnea in a sleep laboratory and 78 control subjects were examined. Peripheral blood samples were taken from patients diagnosed with obstructive sleep apnea by polysomnography. DNA was extracted from blood samples and amplified using polymerase chain reaction. Amplification products were digested with restriction enzymes to investigate gene polymorphisms. Restriction products were extracted from agarose gel electrophoresis and polymorphisms were analyzed using gel images. The Arg16Gly polymorphism was observed in 18 of 52 patients and in 23 of 78 controls. The Gln27Glu polymorphism was observed in 21 of 52 patients and in 28 of 78 controls. In conclusion, there was no correlation among polymorphic

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frequencies between patient and control groups. Based on the results, these polymorphisms do not contribute to the clinical diagnosis of this syndrome. However, the distribution of Arg16Gly *vs* Gln27Glu polymorphisms may contribute to obesity in patients with a body mass index greater than 30 ($P < 0.05$). Different results may be obtained if the parameters of obstructive sleep apnea disease are changed.

Key words: *ADRB2* gene polymorphisms; Body mass index; Obesity; Restriction fragment length polymorphism; Turkish population; Obstructive sleep apnea syndrome

INTRODUCTION

Sleeping, which is an indispensable necessity for human health, serves to biologically and psychologically regenerate an organism. Sleeplessness can trigger various illnesses and weakens the labor force, contributing to overall economic losses (Punjabi, 2008). Obstructive sleep apnea syndrome (OSAS) is defined as diurnal sleepness that occur during sleep because of repeated obstructions in the upper airway (Kaparianos et al., 2006). Apnea is defined as the stoppage of airflow through the mouth and nose for a duration of 10 s or more. The clinical diagnosis of OSAS is related to several metabolic diseases such as, hypertension, diabetes, endocrine diseases, and obesity (Prentice, 2006). In recent years, progress has been made in diagnosing this disease using polysomnography (PSG) tests (Mokhlesi and Gozal, 2011).

In different countries, OSAS differs in terms of clinical findings, and the prevalence has been shown to increase in individuals 40-65 years of age (Ge et al., 2005; Zhang et al., 2005). The incidence rate of OSAS is 4% in men and 2% in women (Larkin et al., 2010). Approximately 0.9-1.9% of the Turkish population suffers from sleep apnea syndrome (Sengul et al., 2011). In the USA, 12 million people aged 30-65 years have OSAS, and approximately 25% have medium or severe apnea according to the Apnea Hypopnea Index (AHI) (Daghestani et al., 2012; Herr et al., 2013). In a study conducted in the USA, Young et al. (2003) examined continuous snoring in 1453 individuals 30-60 years of age who appeared to be healthy. The researchers applied PSG to 602 subjects and found AHI values of >5 in 9% of women and 24% of men, AHI > 10 in 5% of women and 15% of men, and AHI > 20 in 4% of women and 9% of men (Young et al., 2003; Daghestani et al., 2012).

Adrenergic receptor genes, which are responsible for the formation of respiration-related illnesses, as well as the variety of polymorphisms observed in these genes may be used as diagnostic criteria for OSAS (Schwartz et al., 2008). In addition, increasing body mass index (BMI) is related to increases in the diversity of adrenergic gene polymorphisms. Some genomic studies have shown that the prevalence of OSAS is higher among families and in twins (Fragoso et al., 2006). The β-2 adrenergic gene receptors (ADRB2) are synthesized in the body in various cells that are targets of adrenaline and noradrenalin and are involved in regulating the cardiac, pulmonary, vascular, and central nerve systems (Fragoso et al., 2006). The β-2 adrenergic receptor is encoded by the *ADRB2* gene, which contains no introns and has been localized to chromosome 5q33.1. These polymorphism regions were found to be Arg16Gly and Gln27Glu *in vitro* (Larkin et al., 2010). In a Scandinavian study, it was found that the *ADRB2* gene is related to hypertension, respiratory passage diseases, obesity, heart disease, and diabetes (Kalra and Chakraborty, 2008).

Obesity, which primarily results from nutrition habits, is one of the most important problems currently threatening human health and has been defined as "the secret illness" (Daghestani et al., 2012). Studies in the USA have predicted that together with increasing obesity, OSAS will become a major health problem (Young et al., 2003; Bartels et al., 2007). We selected a northeastern region in Turkey for this study because the prevalence of heart and respiratory diseases and obesity related to the culture's nutrition is higher than the national average. In this study, we examined the prevalence of *ADRB2* Arg16Gly and Gln27Glu polymorphisms in this region using molecular techniques on a genomic level in patients diagnosed with OSAS. We determined the prevalence of the polymorphism using the genomic polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) technique.

MATERIAL AND METHODS

Patients and population

In this study, 52 of 800 subjects diagnosed with OSAS were studied at the sleep laboratory at Kafkas University Medicine Faculty Research and Application Hospital from 2011-2013. A control group of 78 subjects with an AHI ≥ 5 were selected randomly from the population. The population in this region shows trends toward obesity resulting from poor nutrition habits and the long winter season. In addition, sleep disease diagnoses in the region exceed the country's average. The demographic distribution of patients and controls is shown in Table 1 ($P < 0.05$).

Table 1. Demographic characteristic of obstructive sleep apnea syndrome (OSAS) patients and control group.

OSAS patients (AHI < 30) (N = 52)					Control group (AHI > 5) (N = 78)					P
Age	Female (N)	%	Male (N)	%	Age	Female (N)	%	Male (N)	%	
19-41	4	7.6	12	23	19-41	15	19.2	22	28.2	0.039
42-60	11	21.0	17	32.7	42-60	14	18.1	19	24.3	0.009
>61	4	7.6	4	7.7	>61	4	5.2	4	5.2	0.235
Total	19	36.5	33	63.5	Total	33	42.3	45	57.7	

Study procedure

OSAS is a condition characterized by repetitive episodes of apnea and hypopnea during sleep because of airway obstruction. Its main symptoms include loud snoring, frequent awakenings, disrupted sleep, excessive daytime sleepiness, fatigue, and decreased cognitive abilities (Mannarino et al., 2012). Full-night polysomnographic recording was performed using an Embla N7000 system (Medcare; Reykjavik, Iceland). The following parameters were recorded: electroencephalography, electrocardiography, electrooculography, submental and anterior tibialis muscle electromyography, nasal pressure, oronasal airflow by thermal sensor, snoring, oxygen saturation by finger oxymeter, and respiratory effort by thoracic and abdominal inductance plethysmography. Sleep disordered breathing events were scored manually by the same investigator, N. Huseyinoglu, according to the American Academy of Sleep Medicine criteria (Iber et al., 2007). Obstructive apnea was defined as a drop in the peak oronasal thermal sensor excursion by ≥90% from baseline for at least 10 s. Hypopnea was defined as

at least a 50% drop in airflow for at least 10 s despite respiratory efforts and at least a 3% decrease in oxyhemoglobin saturation. Patients were diagnosed with OSAS if the AHI was ≥5. Grading was conducted according to mild OSAS with an AHI of 5-15, moderate OSAS with an AHI of 15-30, and severe OSAS with an AHI ≥ 30 (Iber et al., 2007). The lowest O_2 saturation value was measured throughout the night for each patient. PSG of the patients and controls were recorded in the hospital sleep laboratory using the 24-hour duration as a baseline with the laboratory PSG system (Embla N7000). The heights of the patients and controls were measured in cm, while weights and BMI values were calculated in kg/m^2.

Genotyping

From each patient and control, 8 mL blood samples were collected and DNA was isolated from each sample using the salting-out method (Rapley and Walker, 2008). Isolated DNA samples were stored at -80°C after measuring the concentration using a NanoDrop spectrophotometer (ND1000; Thermo Scientific; Waltham, MA, USA). The primers used to identify *ADRB2* (Arg16Gly) were F: 5'-GCCTTCTTGCTGGCACGCAAT-3' and R: 5'-CCAGTGAAGTGATGAAGTAGTT-3' and the *ADRB2* (Gln27Glu)-specific primers were: F: 5'-CCGGACCACGACGTCACCCAG-3' and R: 5'-CCGGACCACGACGTCACCCAG-3' (Bengtsson et al., 2010). Two 25-µL PCR mixtures were prepared for *ADRB2* Arg16Gly and *ADRB2* Gln27Glu to amplify genes in the DNA samples. The mixtures contained 2.5 µL 10X Taq polymerase buffer, 2 µL 2.5 mM dNTP mixture, 1.25 µL 2.5 mM $MgCl_2$, 1.25 µL 10 pmol of each primer, 0.2 µL 5 U Taq polymerase (Bioron; Ludwigshafen, Germany), 1 µL 50 ng DNA, and ddH_2O. *ADRB2* Arg16Gly and *ADRB2* Gln27Glu PCR conditions were as follows: initial denaturation for 3 min at 95°C, followed by 30 cycles at 94°C for 30 s, 60°C for 30 s, 72°C for 45 s, and a final extension at 72°C for 7 min. The PCR products were detected by agarose gel electrophoresis (at 120 V, 300 A for 1.5 h) on a 2% agarose gel containing ethidium bromide, and the fluorescence intensity of each band was evaluated using an ultraviolet transilluminator (Gel Logic 2200 Pro; Carestream Health; Rochester, NY, USA). For the *ADRB2*-Arg16Gly polymorphism, PCR amplification bands were observed as 200 and 160 bp, indicating the presence of an *ADRB2*-Gln27Glu PCR amplification product. Amplified products were digested using 2 U *Bacillus stearothermophilus* (*Bst*DI) for *ADRB2*-Arg16Gly and *B. stearothermophilus* (*Bst*NI) for *ADRB2*-Gln27Glu (New England Biolabs; Ipswich, MA, USA) (Liu et al., 2006). Digestion products were visualized by separating the resulting fragments by 2% agarose gel electrophoresis followed by ethidium bromide staining under ultraviolet illumination. The 200-bp strand was separated into 2 DNA fragments of 145 and 55 bp by the restriction enzyme when a polymorphism was present for *ADRB2*-Arg16Gly. This result was accepted as positive and indicated that arginine had been replaced with glycine. In the 2nd region, the typical 170-bp fragment was separated into 2 DNA fragments of 120 and 50 bp for the *ADRB2*-Gln 27Glu polymorphism. This result was accepted as positive and indicated that glycine had been replaced with glutamine. Genomic studies were conducted at the Bioengineering research laboratory, Faculty of Engineering and Architecture, Kafkas University.

Statistical analysis

Statistical analyses were conducted using the GraphPad Prism 6 package program (GraphPad; San Diego, CA, USA). In addition to the descriptive statistical methods, the mean,

standard deviation, and the independent *t*-test were used to compare paired groups, while the Fisher reality test was used to compare qualitative data. A significance level of P = 0.05 was used for all the tests.

RESULTS

The BMIs of patients with OSAS and the control group were calculated and are shown in Table 2 (P < 0.05).

Table 2. Distribution of patients with obstructive sleep apnea syndrome (OSAS) and control group according to gender and body mass index (BMI).

BMI	OSAS patients (BMI) (N = 52)				Control group (BMI) (N = 78)					P
	Female (N)	%	Male (N)	%	BMI	Female (N)	%	Male (N)	%	
20 ≤ 30	3	5.77	17	32.7	20-30	10	12.8	21	26.9	0.068
<31	16	30.77	16	30.76	>31	23	29.5	24	30.8	0.998
Total	19	36.54	33	63.46	Total	33	42.3	45	57.79	

The PSG results of the patients, severity of the illness, and analysis results are shown in Table 3. Genomic analyses in patients with OSAS revealed the Gln27Glu polymorphism in the *ADRB2* gene in 21 (40%) of 52 patients and in 28 (35%) of 78 controls. The *ADRB2* gene Arg16Gly polymorphism was identified in 18 (34%) of 52 patients and in 23 (29%) of 78 controls. The *ADRB2* gene Gln27Glu polymorphism was observed in 10 (48%) women with OSAS and in 11 (52%) men with OSAS, as well as in 12 (43%) women and 16 (57%) men in the control group (P < 0.05) (Tables 4 and 5).

Table 3. Distribution of patients with obstructive sleep apnea syndrome according to gender and the apnea-hypopnea index (AHI).

	Female (N)	%	Male (N)	%	P
5-15 AHI (Slight)	2	3.84	4	7.69	0.424
15-30 AHI (Mild)	4	7.69	11	21.16	0.326
>30 AHI (Severe)	13	25	18	34.61	0.026
Total	19	36.54	33	63.46	

Table 4. Distribution of patients with obstructive sleep apnea syndrome (OSAS) and control Arg16Gly and Gln27Glu gene polymorphisms according to apnea-hypopnea index (AHI).

ADRB2	OSAS patients (AHI 10 ≤ 30)				Control group (AHI < 5)				P value
	Female (N)	%	Male (N)	%	Female (N)	%	Male (N)	%	
Arg16Gly	8	44	10	56	9	39	14	61	0.5675
Gln27Glu	10	48	11	52	12	43	16	57	0.7121

Table 5. Distribution of patients with obstructive sleep apnea syndrome (OSAS) and control Arg16Gly and Gln27Glu gene polymorphisms according to body mass index (BMI).

	BMI > 30 OSAS patients				BMI > 30 control group				P
	Female (N)	%	Male (N)	%	Female (N)	%	Male (N)	%	
Arg16Gly	4	25	14	75	15	34.7	8	65.3	0.0110
Gln27Glu	5	23.8	16	76.2	18	64.2	10	35.8	0.0087

The *ADRB2* Arg16Gly polymorphism was observed in 8 (44%) women with OSAS and 10 (56%) men with OSAS, as well as in 9 (39%) women and 14 (57%) men in the control group. In polymorphism analysis by BMI, the Gln27Glu polymorphism was observed in 21 of 32 subjects whose BMIs were >30 of patients with OSAS and in 28 of 47 subjects whose BMIs were >30 of healthy individuals. The *ADRB2* genotype and allele frequencies are shown in Tables 6 and 7 ($P < 0.05$).

Table 6. Genotypes and allelic frequencies of *ADRB2* gene (Arg16Gly) polymorphisms in patients with obstructive sleep apnea syndrome (OSAS) and controls.

	OSAS patients						Healthy control group						P
	A/A	%	A/G	%	G/G	%	A/A	%	A/G	%	G/G	%	
Men (N = 24)	3	30.00	5	50.00	2	20.00	2	14.28	10	71.42	2	14.28	0.192
Women (N = 17)	2	25.00	5	62.25	1	12.50	2	22.22	6	66.66	1	11.11	0.924
Total	5	27.77	10	55.55	3	16.66	4	17.39	16	69.56	3	13.04	0.272
Allele	0.56				0.44			0.53			0.47		

A = arginine (Arg); G = glycine (Gly); OSAS 10 ≤ 30: subjects = 10-30 events/h by apnea-hypopnea index 11-30 events/h.

Table 7. Genotypes and allelic frequencies *ADRB2* gene (Gln27Glu) polymorphisms in patients with obstructive sleep apnea syndrome (OSAS) and controls.

	OSAS patients						Healthy control group						P
	A/A	%	A/G	%	G/G	%	A/A	%	A/G	%	G/G	%	
Men (N = 28)	4	33.32	6	50.00	2	16.66	4	25.00	10	62.50	2	12.50	0.407
Women (N = 21)	3	33.33	5	55.55	1	11.11	3	13.63	8	81.81	1	4.54	0.0001
Total	7	33.33	11	52.38	3	14.28	7	25.00	18	64.28	3	10.71	0.215
Allele	0.60				0.40		0.62				0.38		

Gln = glutamine; Glu = glutamine acid; OSAS 10-30 subjects = 10-30 events/h by apnea-hypopnea index 11-30 events/h.

DISCUSSION

In recent years, in addition to the clinical diagnostic methods for OSAS, genetic studies have become increasingly important. Thus, we examined the distribution of the Arg16Gly and Gln27Glu polymorphisms in individuals diagnosed with OSAS in the Northeastern Anatolia population. Seventy percent of patients with OSAS included in this study were over 40 years of age, and 62% had a BMI greater than 30. In patients with OSAS, the *ADRB2* gene was not associated with the Arg16Gly and Gln27Glu polymorphisms; however, the prevalence of both polymorphisms in men was higher than that in women in these patients. Large (2003) found that body fat in 50% of 140 women studied was greater than 20 kg and that the Gln27Glu polymorphism was associated with obesity risk. The Arg16Gly polymorphism is associated with β2 adrenergic receptor function in individuals with the Gly16 allele; however, this was not found to be a significant risk factor for obesity (Large, 2003). We found similar results for the Gln27Glu polymorphism; however, we also found that both the Arg16Gly and Gln27Glu polymorphisms are risk factors for obesity.

A study conducted in Saudi Arabia population examined 329 individuals, 109 and 220 of which were men and women, respectively, for the Arg16Gly polymorphism. A total of 32.1% of men in this population had normal weights, 25.6% of men were overweight, and 42.2% were obese. In addition, 36.36% of women had normal weights, 18.18% of women

were overweight, and 45.45% were obese (Daghestani et al., 2012). The genotypes AA, AG, and GG were observed in 54.3, 19.56, and 26.1% of men, respectively. In women, the genotypes AA, AG, and GG were observed in 51, 20, and 29% of women, respectively (Daghestani et al., 2012). The researchers argued that the Arg16Gly polymorphism can be a determinant of the development of obesity in Saudi communities (Daghestani et al., 2012). Our study indicated that both polymorphisms can trigger obesity.

French and Spanish researchers conducted a study in 2000 in Europe to examine a population composed of 419 men and 417 women with a BMI of 33.9 ± 3.3 kg/m^2. In this study, an increase in the BMI of participants with the Gln27Gln genotype of Gln27Glu and Arg16 allele of Arg16Gly was observed. The results of the previous study suggest that the β2 adrenergic receptor gene is associated with obesity in French men (Meirhaeghe et al., 2000; Piérola et al., 2007). In our study, a BMI greater than 30 was observed in 16 (76%) of 21 individuals with OSAS and the Gln27Glu polymorphism and in 10 (78%) of 18 individuals with OSAS and the Arg16Gly polymorphism. The results of the French and Spanish studies were similar to the results of our study.

In a study conducted in Europe in 1998, 156 obese men and a control group of 205 males were examined. The mean BMI was 35.8 ± 0.60 kg/m^2. In the obese group and 26.4 ± 0.30 kg/m^2 in the control group. The Gln/Gln phenotype was observed in 28.2% of obese men, the Gln/Glu phenotype in 55.2%, and the Glu/Glu phenotype in 16.6%. In a control group of 3000 subjects, the Gln/Gln phenotype was observed in 33.7%, the Gln/Glu phenotype in 50.7%, and the Glu/Glu phenotype in 15.62%. The significance level of the association between the polymorphism and obesity was $P > 0.05$. Based on these results, it was suggested that the Gln27Glu genotype of the β2 adrenergic receptor gene is not related to obesity (Börgel et al., 2006; Echwald et al., 2006). By contrast, we found that the β2 adrenergic receptor gene is associated with obesity. We observed that the Gln27Glu and Arg16Gly polymorphisms of the β2 adrenergic receptor gene are not directly related to OSAS; however, in the patients and controls with OSAS and a BMI > 30, the risk of obesity was increased. To determine whether obesity-sensitive genes trigger sleep disorders in OSAS patients, more comprehensive genomic screenings are needed.

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