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The Effect of Different *ApoE* Genotypes and Other Risk Factors on Obstructive Sleep Apnea Syndrome Formation

Farklı ApoE Genotiplerinin ve Diğer Risk Faktörlerinin Obstrüktif Uyku Apne Sendromu Oluşumu Üzerine Etkisi

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Abstract

Objective: Obstructive sleep apnea syndrome (OSAS) is a disorder characterized by partial or complete narrowing of the pharyngeal airway during sleep. In this study it was aimed to investigate the relation between OSAS and different variants of the *ApoE* gene, and to identify other risk factors that may affect the development of the disease.

Materials and Methods: Fifty-two patients with OSAS and 50 healthy volunteers were enrolled into the study. After collecting the necessary information associated with OSAS from the individuals, DNA was isolated from blood. ε2, ε3 and ε4 variants of *Apolipoprotein E (ApoE)* gene were investigated using real-time polymerase chain reaction.

Results: When the groups were compared with each other, age, body mass index, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein, triglyceride, neck circumference, waist circumference, apnea hypopnea index, Epworth sleepiness scale, smoking, and daytime sleepiness were found statistically significant. The ε2 variant was found statistically high in the control group. Also, waist circumference, triglyceride and LDL levels were found statistically low in individuals with the ε2 genotype. In addition, triglyceride levels were found statistically high in individuals with the ε4 genotype.

Conclusion: The presence of the ε2 variant in healthy individuals may have a protective effect against OSAS. In addition, the relation between different variants of *ApoE* with LDL and triglyceride levels demonstrates the overlap of genotype and phenotype data.

Keywords: Obstructive sleep apnea syndrome, *ApoE*, real-time polymerase chain reaction

Öz

Amaç: Obstrüktif uyku apne sendromu (OUAS), uyku esnasında faringeal hava yolunun kısmen veya tamamen daralması ile karakterize olan bir hastalıktır. Bu çalışmada OUAS ile *apolipoprotein E (ApoE)* genindeki çeşitli varyantların ilişkisinin araştırılması ve hastalık oluşumuna etki edebilecek diğer risk faktörlerinin incelenmesi amaçlanmıştır.

Gereç ve Yöntem: Elli iki OUAS hastası ve 50 sağlıklı gönüllü birey çalışmaya dahil edilmiştir. Bireylerden OUAS ile ilişkili olabilecek gerekli bilgiler temin edildikten sonra, kandan DNA izolasyonu yapılmıştır. *ApoE* genindeki ε2, ε3 ve ε4 varyantlarının incelenmesi gerçek zamanlı polimeraz zincir reaksiyonu ile gerçekleştirilmiştir.

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Presented in: This study was titled as ''Effect of *ApoE* Gene Variants on Formation of Obstructive Sleep Apnea Syndrome (OSAS)'' and presented in debatable poster session of 52nd National Neurology Congress.

Bulgular: Gruplar arasında karşılaştırma yapıldığında yaş, vücut kitle indeksi, total kolesterol, düşük yoğunluklu lipoprotein (LDL), yüksek yoğunluklu lipoprotein, trigliserid, boyun çevresi, bel çevresi, apne-hipopne indeksi, Epworth uykululuk ölçeği, sigara kullanımı, gündüz uykusu özelliklerinin istatistiksel açıdan anlamlı olduğu belirlenmiştir. ε2 varyantı kontrol grubunda anlamlı düzeyde yüksek bulunmuştur. ε2 genotipine sahip bireylerde bel çevresi, trigliserid ve LDL değerleri istatistiksel açıdan anlamlı düzeyde düşük olarak bulunmuştur. Ek olarak ε4 genotipine sahip bireylerde trigliserid seviyesi anlamlı düzeyde yüksek saptanmıştır.

Sonuç: Sağlıklı bireylerde ε2 varyantının bulunmasının OUAS için koruyucu etkisi olabilir. Ek olarak *ApoE*'nin farklı varyantlarının, LDL ve trigliserid seviyeleriyle ilişkili olması genotip ve fenotip verilerinin örtüştüğünü göstermektedir.

Anahtar Kelimeler: Obstrüktif uyku apne sendromu, *ApoE*, gerçek zamanlı polimeraz zincir reaksiyonu

Introduction

Obstructive sleep apnea syndrome (OSAS) is a complex and chronic disease with repeated episodes of recurrent collapse of the upper airway during sleep, which are characterized by significant decrease in airflow (hypopnea) despite continued breathing or complete cessation (apnea). These episodes can result in oxygen desaturation, day and night catecholamine fluctuations, intrathoracic pressure changes, disruption of sleep, and excessive daytime sleepiness (1,2).

It was estimated that obstructive sleep apnea associated with excessive daytime sleepiness affected 2% of middle-aged women and 4% of middle-aged men in the United States in the 1990's (3,4). The prevalence of OSAS in Turkey was found as 0.9-1.9% (5) in one study, and 13.7% in another study in 5521 individuals (6).

The most important risk factors for OSAS are defined as obesity, male sex, short neck structure, craniofacial anomalies, advanced age, smoking, and alcohol use (4,7,8,9). In addition, patients with OSAS have been shown to have a high risk of morbidity and mortality due to cardiovascular and cerebrovascular diseases (9). Studies have indicated that men are at higher risk of developing OSAS than women (10). Obesity is considered to be the most important of these factors because it is found in approximately 70% of patients with OSAS. Especially in developed countries, this rate reaches epidemic proportions and it is the only reversible factor among related risk factors. However, the mechanisms that explain the relationship between obesity and OSAS have not been fully identified (4).

Apolipoprotein E (ApoE) is a 299-amino acid macromolecule-structured plasma protein found in senile plaques, vascular amyloid (β-amyloid), and neurofibrillary tangles in Alzheimer's disease. The *ApoE* gene, encoding the *ApoE* protein, is localized on the 19q13.2 chromosomal region and contains 4 exons. The 3 alleles of the gene are named as ε2, ε3, and ε4 (11,12). The ε3/ε3 phenotype is the most commonly found phenotype caused by these alleles (11,13).

ApoE is a plasma protein that plays a key role in lipoprotein homeostasis and the lipoprotein transport system (14). It is one of the plasma lipoproteins that has about a dozen protein components and is involved in the structural continuity of lipoprotein particles, and the regulation of several different lipoprotein metabolisms (15). *ApoE* isoforms can bind differently to β-amyloid and tau proteins, leading to neurofibrillary tangles and plaques in the cerebral respiratory control center, and have potential effects on ventilation stability during sleep (14).

ApoE is a plasma protein that mediates high-affinity receptor binding of lipoproteins to the low-density lipoprotein (LDL) receptor. It is known that *ApoE* has functions such as differentiation, cell growth, and regulation of the immune system, and that it is expressed in various organs such as the spleen, kidney, liver, and brain (16,17). *ApoE* has been found at high concentrations in the interstitial fluid, which is responsible for the redistribution of cholesterol to cells with low cholesterol from cells with excessively high cholesterol (17). Currently, the specific functions of *ApoE* enzymatic activity in the brain and the mechanisms mediating *ApoE*-related neuroprotection against neurodegenerative processes are still unknown (18).

Today, it is known that genetic factors play a role in the formation of OSAS, as well as environmental factors. In this study, it was aimed to investigate the distribution of ε2, ε3, and ε4 variants of *ApoE* gene, which are thought to cause OSAS, in patients with OSAS and healthy individuals, and to investigate the relation of these variants with various environmental factors.

Materials and Methods

Creating Study Groups

The study included 52 male patients who were diagnosed as having OSAS with an apnea-hypopnea index (AHI) score of ≥5, and 50 healthy male volunteers with an AHI score of <5 as a result of whole-night polysomnography performed in the Sleep Diagnosis and Treatment Center, at Erenköy Mental and Neurological Diseases Training and Research Hospital, Health Sciences University.

Whole-night polysomnography examination was performed using a Neurosoft (Neurosoft, Ivanovo, Russia) Polysomnography System with 6-channel electroencephalography (C4-A1, C3-A2, 02-A1, 01-A2, F4-A1 F3-A2), 2-channel electrooculography, chin electromyography (EMG), right and left anterior tibial EMG, body position, an oro-nasal thermal sensor, nasal pressure sensor, thoracic and abdominal respiratory movement tapes, electrocardiography, respiratory sound recording, O_2 saturation, and synchronous video recording.

Sleep-related abnormal respiratory events were scored by a neurologist (FMD), who has knowledge and experience in sleep disorders, in accordance with the American Academy of Sleep Medicine (2014) criteria.

Apnea was considered when the following criteria were met: peak signal excursions drop by ≥90% of pre-event baseline using thermal sensor, the duration of the respiratory event was ≥10 seconds, and at least 90% of these events fulfilled drop criteria.

Hypopnea was considered when there was a ≥30% drop in the peak signal excursions of pre-event baseline using nasal cannula, the duration of the drop in signal excursion was ≥10 seconds, and ≥3% oxygen desaturation from pre-event baseline and/or the event was associated with an arousal (19). Based on the AHI, the severity of OSAS was classified as follows: mild, between 5-14; moderate, between 15-29; and severe, ≥30.

The Epworth sleepiness scale (ESS) test was performed in all subjects. Total cholesterol, LDL cholesterol, high-density lipoprotein cholesterol (HDL), and triglyceride levels of the patient and control groups were examined. Patients who were considered for surgery as a result of otorhinolaryngologic examination, and in order to exclude the obesity factor, individuals with a body mass index (BMI) value greater than 30 kg/m^2 were excluded from the study.

The study, which is compatible with the Helsinki Declaration, was approved by the Yeditepe University Ethics Committee of Clinical Investigations of (Date: 22.06.2015, Decision no: 62/495). Each individual was informed about the study and written informed consent was obtained.

Blood Sample Collection and Genotyping Studies

The genomic DNA was isolated from 200 µL blood samples using a DNA isolation kit (Roche) according to the manufacturer's instructions. DNA purity and concentration were measured using a NanoDrop spectrophotometer (Thermo Scientific). Using a real-time polymerase chain reaction device, the ε2, ε3, and ε4 variances in the *ApoE* gene were examined from the obtained DNA (Applied Biosystems). The *ApoE* ε2, ε3, and ε4 variants correspond to the *ApoE* SNP haplotypes T-T, T-C, and C-C in rs429358 and rs7412, respectively. "T" allele in rs7412 and "T" allele in rs429358 represent ε2 variant, "C" allele in rs7412 and "T" allele in rs429358 represent ε3 variant, and "C" allele in rs429358 and "C" allele in rs7412 represent ε4 variant (20). The ε3 variant is the most common isoform formed by the coding of cysteine at codon 112 and arginine amino acid at codon 158. The ε2 variant is formed by coding of cysteine at codon 112 and cysteine amino acid at codon 158. The ε4 variant is formed by coding of arginine at codon 112 and arginine amino acid at codon 158 (16,21). The experiments were conducted in accordance with the protocol of the relevant company.

Statistical Analysis

The data were analyzed on a computer using the Statistical Packages of Social Sciences (SPSS) version 24.0 software. The Kolmogorov-Smirnov test was used to test for the normality of data. Descriptive statistics are shown as mean ± standard deviation (SD) for continuous variables, and frequency and percentage for categorical variables. The independent two-sample t-test was used to compare the normally distributed data of two independent groups. The chi-square test or Fisher's exact test was used for the analysis of the difference between the categorical variables. The Kruskal-Wallis test was used to compare variables of more than two groups that did not fit normal distribution, and the Mann-Whitney U test was used for pairwise comparisons. The results were interpreted after performing Bonferroni correction. The contingency coefficient was calculated to determine whether there was a relationship between mutations. A p value <0.05 was considered significant.

Results

Study Groups

Regarding intergroup comparisons, it was determined that age, BMI, total cholesterol, LDL, HDL, triglyceride, neck circumference, waist circumference, AHI, ESS, smoking, and daytime sleepiness were statistically significant ($p<0.05$). There was no statistical significance for the presence of hypertension and coronary artery disease. Alcohol use and stroke were not evaluated because they were not found in any individuals. Table 1 shows the characteristics of the individuals who participated in the study.

ε2, ε3, and ε4 Genotyping

The ε2 variance in the *ApoE* gene was statistically significantly higher in the control group ($p<0.05$). No statistical significance was found for ε3. Although the ε4 variance was higher in the patient group compared with the control group, this difference was not statistically significant. Table 2 gives the genotype distributions of the groups.

The Relationship Between Risk Factors and *ApoE* Gene Mutations

Triglyceride levels were significantly higher in individuals with the ε4 allele ($p<0.05$). Triglyceride, LDL, and waist circumference were significantly lower in individuals with the ε2 allele ($p<0.05$). The significance of risk factors with genotypes is summarized in Table 3.

Discussion

It is known that the majority of sleep disorders are the result of a complex interaction between environmental factors and individual genetic predisposition (21). To date, 85 genes have been listed as OUAS susceptibility genes. Among these, the presence of gene alleles in the top three positions that encode *ApoE* is striking (22). For this reason, most current studies have focused on the *ApoE* ε2, ε3, and ε4 variants to investigate disease-related genetic factors. The ε4 allele in particular is thought to be an effective factor in disease progression (3,16,18). In some studies, no association was found between *ApoE* gene variants and sleep apnea syndrome (23). The fact that the ε2 allele in our study was statistically significantly higher in the control group suggests that this allele may have a protective effect against the disease. We believe that the ε4 variant may be related to the disease formation because the ε4 variant was detected at a high rate in the patient group compared with the controls. However, because the difference between the two groups was not statistically significant, we think that it would be useful to investigate the effect of ε4 variant on OSAS formation by increasing the number of patients.

In various studies, it was determined that *ApoE* variants affected lipid profile, the ε2 variant was associated with low total cholesterol and LDL levels, and the ε4 variant was associated with high total cholesterol and LDL levels (24,25). It has been reported that the ε2 and ε4 alleles are also associated with triglyceride levels. It is also known that related mutations affect the plasma concentration of *ApoE* (25,26,27). However, the role of *ApoE* mutations in cholesterol and triglyceride metabolism has not been fully elucidated. When we investigated the relationship between the risk factors for OSAS and *ApoE* variants in our study, similar

results were obtained with other studies; waist circumference, triglyceride, and LDL levels were found significantly lower in subjects with the ε2 allele compared with other *ApoE* variants. Triglyceride levels in individuals with ε4 alleles were significantly higher. No relationship was found between the genotypes and age, BMI, total cholesterol, HDL, neck circumference, AHI, ESS, smoking, daytime sleepiness, hypertension, and coronary artery disease in our study. In one study, the mean BMI and AHI were compared among individuals who had one or more *ApoE*-ε4 alleles and who did not have any alleles, and similar with our findings, no difference was determined between the groups (28). In another study, increased AHI was observed in patients with OSAS with ε3/ε4 genotypes. In the same study, although ε3/ε4 genotypes were found to be significant in terms of hypertension, there was no significant relationship between genotypes and sex, smoking, BMI, and alcohol use parameters (29). In another study, an association was determined between ESS and the rs429358 mutation of the *ApoE* gene, but this relationship was not considered strong enough to be clinically valuable in terms of ESS (30). In our study, ESS values were found to be high in individuals with ε4 alleles, but this difference was not significant.

In our study, an intergroup comparison revealed that AHI, ESS, BMI, neck circumference, waist circumference and total cholesterol, and triglyceride and LDL levels associated with lipid metabolism were significantly higher, and HDL levels were significantly lower in patients with OSAS. It was expected that the AHI value, and similarly the ESS value, would be significantly higher in the patient group because the study groups were formed according to the AHI value being below or above 5.

Obesity is known to be a powerful marker for OSAS. An increase of 1 SD in BMI is associated with a four-fold increase in the risk of OSAS (29). In our study, it was also determined that individuals in the OSAS group were generally overweight (25 <BMI <30). In one study, it was found that obesity was significantly correlated with total serum cholesterol levels in men and HDL and triglyceride levels in women, as well as respiratory disturbance index in both (31). Therefore, it is thought that the results obtained from the intergroup comparison of the risk factors in our study are similar to the literature.

Several studies have determined that age can also be an effective factor in the process of OSAS formation (32). Based on laboratory criteria, it has been observed in the past that the prevalence of sleep apnea is higher in older people than in young people. In a comprehensive series, Kripke et al. (30) observed a prevalence of sleep apnea ranging between 24% and 62% in the elderly population. In a study performed in our country, female sex, older

Table 1. Characteristics of the study population

Characteristics	Mean values and distributions		p value
	Control group (n=50)	Patient group (n=52)	
Age (years)	42.86±8.54	46.5±9.82	**0.048***
BMI (kg/m²)	21.91±1.95	27.24±2.26	**<0.001****
Total cholesterol (mg/dL)	180.7±38.62	207.48±41.67	**0.001***
LDL (mg/dL)	101.63±30.8	123.4±32.49	**0.001***
HDL (mg/dL)	64.84±20.74	42.13±10.96	**<0.001****
Triglyceride (mg/dL)	71.35±31.69	207.9±125.33	**<0.001****
Neck circumference (cm)	38.62±2.17	40.77±3.04	**<0.001****
Waist circumference (cm)	72.46±8.06	103.65±8.17	**<0.001****
AHI (per hour)	2.56±0.83	36.16±19.37	**<0.001****
ESS	2.28±0.904	5.65±5.27	**<0.001****
Smoking	3 (6%)	18 (34.6%)	**<0.001****
Daytime sleepiness	0 (0%)	26 (50%)	**<0.001****
Hypertension	1 (2%)	5 (9.6%)	0.205
CAH	0 (0%)	4 (7.7%)	0.118
Alcohol use	0 (0%)	0 (0%)	NE
History of stroke	0 (%0)	0 (%0)	NE

BMI: Body mass index, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, AHI: Apnea-hypopnea index, ESS: Epworth Sleepiness Scale, CAH: Coronary artery disease, p<0.05*, p<0.001**, NE: Not evaluated

Table 2. Genotype distributions of groups

Gene, genotype variation	Distributions		p value
	Control group (n=50)	Patient group (n=52)	
ApoE			
ε2	15 (30%)	4 (7.7%)	**0.005***
ε3	30 (60%)	37 (71.1%)	0.236
ε4	5 (10%)	11 (21.2%)	0.122

*p<0.05, *ApoE: Apolipoprotein E*

Table 3. Relationship between statistically significant risk factors and *ApoE* mutations

The relationship between risk factor and mutations	Mean values and distributions		p value
ε2	Absent	Present	
Triglyceride (mg/dL)	147.79±115.43	111.16±108.59	**0.044***
LDL (mg/dL)	118.09±33.23	89.32±22.48	**0.001***
Waist circumference (cm)	90.3±17.11	78.89±17.82	**0.025***
ε4	Absent	Present	
Triglyceride (mg/dL)	113.37±114.39	181.81±110.05	**0.044***

p<0.05*, LDL: Low-density lipoprotein

age, lower educational level, smoking, and age were determined to be associated with risk of sleep disorders (9). Regarding these findings, it is thought that there is a close relationship between age and sleep apnea. Although the Wisconsin Sleep Cohort Study demonstrated a two-fold increase in OSAS in patients with the ε4 allele compared with those without this allele among middle-aged adult participants, no such relationship was observed among older participants in the Honolulu-Asia Aging Study (3). The individuals who participated in our study were mostly middle aged.

Smoking is a susceptibility factor for pulmonary and cardiovascular diseases, and is considered a risk factor for OSAS development. Current studies show a synergistic effect between OSAS and smoking that increases the risk of cardiovascular disease by oxidative stress, endothelial dysfunction, and abnormal inflammatory response. It is also acknowledged that OSAS is responsible for nicotine addiction. It is thought that smoking is an independent risk factor for snoring and may be associated with sleep apnea because snoring is often seen in smokers and is a common symptom even in preclinical cases of OSAS (33). Statistically significant snoring in smokers and daytime sleepiness in the patient group in our study are consistent with data obtained from other studies.

Conclusion

As a result of the present study, the presence of significantly more ε2 variants in the control group than in the OSAS group suggests that this variant may have a protective role in the disease process. Although the frequency of ε4 variants was high in the patient group, and it might be related to disease occurrence, it would be useful to further investigate the relationship by increasing the number of patients because the difference between the two groups was not statistically significant.

ApoE is a protein found in the lipoprotein structure and is known to be associated with serum lipid levels. In our study, different variants of *ApoE* were also found to be closely related to LDL and triglyceride levels, which are associated with lipid metabolism.

The factors that cause OSAS are still not fully clarified at present, so our findings should be supported by other studies.

Ethics

Ethics Committee Approval: The present study protocol conforms to the Declaration of Helsinki and was approved by the Institutional Ethics Committee of Yeditepe University, Istanbul, Turkey (Protocol number: 62/495).

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.F.M.D., Ö.Y.Y., Concept: D.K., S.F.M.D., E.G., Design: D.K., S.F.M.D., E.G., Data Collection or Processing: S.F.M.D., Ö.Y.Y., H.G., D.K., Analysis or Interpretation: E.Ç.A., D.K., S.F.M.D., E.G., Literature Search: D.K., H.G., S.F.M.D., Writing: D.K., H.G., S.F.M.D., E.G.

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