The Relationship between Obstructive Sleep Apnea Syndrome and Apolipoprotein E Genetic Variants

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Key Words
Obstructive sleep apnea syndrome · Apolipoprotein E · Gene polymorphism

Abstract
Background: Clinical and epidemiological studies indicate that obstructive sleep apnea syndrome (OSAS) has a strong genetic basis. Objectives: To investigate the apolipoprotein E (APOE) alleles as a genetic risk factor in OSAS. Methods: A total of 73 patients (37 male) were included. All underwent full-night polysomnography and were evaluated for APOE alleles. Results: The mean age was 51 ± 12 years. Forty-two of the patients had OSAS. The APOE3 allele was found in 97.3\% (71/73) of the study population. The most common APOE genotype was E3/E3 (55/73, 75.3\%). Compared to the individuals with no APOE2 alleles (E3/E3, E3/E4), the individuals with at least one APOE2 allele (E2/E3, E2/E4) had a 9.37-fold greater OSAS risk (OR = 9.37, 95\% CI 1.13–77.7, p = 0.019). The individuals with APOE2 alleles (E2/E3, E2/E4) compared to the individuals with only an E3/E3 allele genotype had a 10-fold greater OSAS risk (OR = 10.3, 95\% CI 1.24–86.61, p = 0.0308). Compared to the individuals with no APOE4 alleles (E2/E3, E3/E3), the individuals with APOE4 alleles (E2/E4, E3/E4) had a high but insignificant risk for OSAS (OR = 2.9, 95\% CI 0.55–15.05, p = 0.286). The individuals with APOE4 alleles (E2/E4, E3/E4) compared to APOE3 alleles (E3/E3) had an increased but insignificant risk for OSAS (OR = 3.62, 95\% CI 0.96–19.05, p = 0.127). Conclusion: Specific APOE genotypes are associated with OSAS in a high-risk population.

Introduction
Obstructive sleep apnea syndrome (OSAS) is characterized by repetitive, complete or partial collapse of the pharyngeal airway during sleep and a general reduction in oxygen desaturation \cite{1}. OSAS is a prevalent disorder, affecting approximately 4–9\% of middle-aged adults \cite{2}. Racial studies and chromosomal mapping, familial studies and twin studies have provided evidence for the possible link between OSAS and genetic factors \cite{3}. It is suggested that most of the risk factors involved in the pathogenesis of OSAS are largely genetically determined \cite{3}.

There is a very high prevalence of OSAS in obese individuals. Similarly, a high prevalence of obesity is observed in patients with OSAS. In one study, the prevalence of OSAS was 60.4\% in morbidly obese patients, and in 33.7\% it was of a severe degree \cite{4}. The pathophysiology of OSAS is intimately linked to obesity. Anatomic and func-
tional considerations of the pharyngeal airway, the central nerve system, central obesity and leptin likely interact in the development of OSAS in obese individuals. Another factor in obese individuals is the distribution of fat. An increased waist circumference can lead to OSAS, even in nonobese individuals [5,6]. The prevalence of OSAS in persons with a BMI < 25 is much less than in higher BMI categories [7]. Most of the patients with OSAS have a short and thick neck. A neck circumference in males and females over 43 and 38 cm, respectively, is an important risk factor for OSAS [8].

Apolipoprotein E (APOE) is a plasma protein that serves as a ligand for low-density lipoprotein receptors and, through its interaction with these receptors, participates in the transport of cholesterol and other lipids among various cells of the body. APOE is synthesized in various organs, including the liver, brain, spleen and kidney, and is present in high concentrations in interstitial fluid, where it appears to participate in cholesterol redistribution from cells with excess cholesterol to those requiring cholesterol. APOE also appears to be involved in the repair response to tissue injury; for example, markedly increased amounts of APOE are found at sites of peripheral nerve injury and regeneration [9–11].

The APOE gene is mapped to chromosome 19 in a cluster with APOC1 and APOC2. APOE is a polymorphic protein arising from three alleles at a single gene locus. APOE is polymorphic with three major isoforms: APOE2 (cys112, cys158), APOE3 (cys112, arg158) and APOE4 (arg12, arg158). Although these allelic forms differ from each other by only one or two amino acids at positions 112 and 158, these differences alter the APOE structure and function. There are 6 genotypes of APOE: E2/E2, E2/E3, E3/E3, E2/E4, E3/E4 and E4/E4 [11–13]. Their frequencies vary substantially around the world, but APOE3 is the most common almost everywhere and is often considered to be the ancestral or 'wild-type' allele for that reason [14]. The E4 variant is the largest known genetic risk factor for late-onset familial and sporadic Alzheimer disease in a variety of ethnic groups [15]. The E4 variant is also reported to be related with the progression and severity of OSAS [16], diabetic neuropathy [17], multiple sclerosis [18], amyotrophic lateral sclerosis [19], cognitive function disorder after coronary bypass surgery [20] and head trauma [21].

There have been few studies investigating the possible relationship between OSAS and APOE. In this study, we aimed to investigate the APOE alleles as genetic risk factors for OSAS.

Methods

Study Group

The study is a prospective trial. A total of 73 consecutive adult patients who attended the Department of Chest Diseases, School of Medicine, Düzce University, between October 2006 and May 2009 were included. All of these patients underwent a polysomnographic examination. Written informed consent was obtained from all participants, and the local ethical committee approved the trial.

Study Design

Prior to the sleep test, all patients filled out a questionnaire regarding sleep disturbance. Using that form, basic OSAS symptoms such as snoring (presence of snoring for at least 5 nights per week), witnessed apnea (spouse or relatives of patients with OSAS identifying noisy and irregular snoring or stopped respiration through the mouth and nose) and daytime sleepiness were evaluated. The Epworth Sleepiness Scale was used to objectively evaluate excessive daytime sleepiness. If the score obtained on this scale was above 10, excessive daytime sleepiness was considered [22].

After evaluating all patients included in the study, the patients were called for an overnight polysomnography (PSG) test with an appointment at the sleep laboratory. PSG (Somnoscreen-PSG, No. 0372 CAA5-O; Somno-Medics, Kist, Germany), electroencephalography, electrooculography, chin electromyography, oral and nasal air flow (nasal-oral 'thermistor' and nasal cannula), thorax movements, abdominal movements, arterial oxygen saturation (pulse oximetry), ECG and snoring recordings (>6 h) were obtained. All records were scored manually using a computer.

Laboratory Analysis

In order to determine gene mutations, DNA isolation was performed from peripheral blood samples. APOE2, E3 and E4 alleles were determined by a polymerase chain reaction and reverse hybridization (ViennaLab strip assay; ViennaLab, Vienna, Austria). Blood samples of patients and controls were collected in 2-ml EDTA tubes. The APOE gene polymorphisms examined were APOE2 (cys112, cys158), APOE3 (cys112, arg158) and APOE4 (arg12, arg158). Although these allelic forms differ from each other by only one or two amino acids at positions 112 and 158, these differences alter the APOE structure and function. There are 6 genotypes of APOE: E2/E2, E2/E3, E3/E3, E2/E4, E3/E4 and E4/E4 [11–13]. Their frequencies vary substantially around the world, but APOE3 is the most common almost everywhere and is often considered to be the ancestral or ‘wild-type’ allele for that reason [14]. The E4 variant is the largest known genetic risk factor for late-onset familial and sporadic Alzheimer disease in a variety of ethnic groups [15]. The E4 variant is also reported to be related with the progression and severity of OSAS [16], diabetic neuropathy [17], multiple sclerosis [18], amyotrophic lateral sclerosis [19], cognitive function disorder after coronary bypass surgery [20] and head trauma [21].

Definitions

The following definitions were used for the terms of interest in this study [23].

Apnea. Complete lack of air flow through the mouth and nose for 10 s or more.
Hypopnea. At least a 50% reduction in the air flow for 10 s or more, along with a 3% decrease in oxygen saturation or arousal from sleep.

Apnea-Hypopnea Index (AHI). The ratio obtained by dividing the total duration of apnea and hypopnea observed during sleep, by the total length of sleep.

OSAS Severity. When evaluated based on AHI, the severity of OSAS was considered as follows: normal (AHI <5); mild sleep apnea (AHI 5–15); moderate sleep apnea (AHI 16–30) and severe sleep apnea (AHI >30).

Statistical Analysis
The subjects were divided into an OSAS and a non-OSAS group. The data were analyzed using SPSS 16.0 (SPSS for Windows; SPSS Inc., Chicago, Ill., USA). The Student t test was used for the paired comparisons of numerical data. The χ² test (Fisher’s Exact Test) was used to compare categorical data.

Odds ratio (OR) was used to determine the relationship between OSAS and different alleles of the apolipoprotein gene. A p value <0.05 was considered to be statistically significant.

Results
Seventy-three consecutive patients (37 male/36 female) with a suspected diagnosis of OSAS were included in the study. The mean age was 51 ± 12 years. According to PSG results, 31 cases had no OSAS. Mild, moderate and severe OSAS was detected in 18, 10 and 14 patients, respectively.

The mean age was statistically low in patients with non-OSAS (p = 0.001). No significant difference was determined between patients with and without OSAS regarding gender, comorbidity, smoking and alcohol habit, level of cholesterol and triglyceride (tables 1, 2). The Epworth Sleepiness Scale and AHI was found to be statistically more significant in patients with OSAS (p = 0.001 and p < 0.001, respectively; table 1). The major symptoms of OSAS (snoring, daytime sleepiness and witnessed apnea) were found to be statistically significant in patients with OSAS (p = 0.001, p < 0.001 and p < 0.001, respectively; table 2). There was at least one major symptom in all patients with OSAS, and a correlation between the frequency of major symptoms and the incidence of OSAS was apparent (table 2).

The APOE genotypes are summarized in table 3. E3 was found in 97.3% (n = 71/73) of the study population. The most common APOE genotype was the E3/E3 genotype (55/73, 75.3%). No APOE E2/E2 or E4/E4 genotypes were present (table 3). The individuals with at least one APOE2 allele (E2/E3, E2/E4) compared to the individuals with no APOE2 alleles (E3/E3, E3/E4) had a 9.37-fold greater OSAS risk (OR = 9.37, 95% CI 1.13–77.7, p =

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Table 1. Patient clinical characteristics

<table>
<thead>
<tr>
<th>Clinical characteristic</th>
<th>Non-OSAS (AHI &lt;5; n = 31)</th>
<th>OSAS (AHI ≥5; n = 42)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>44±13</td>
<td>54±10</td>
<td>0.001</td>
</tr>
<tr>
<td>Smoking, packet years</td>
<td>10±11</td>
<td>14±21</td>
<td>0.282</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>31.8±6.5</td>
<td>35.0±8</td>
<td>0.124</td>
</tr>
<tr>
<td>Epworth sleepiness scale</td>
<td>7±5</td>
<td>12±5</td>
<td>0.001</td>
</tr>
<tr>
<td>AHI</td>
<td>2.2±1.2</td>
<td>31.3±26.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglyceride, mg/dl</td>
<td>172±100</td>
<td>199±99</td>
<td>0.319</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>178±31</td>
<td>190±30</td>
<td>0.177</td>
</tr>
<tr>
<td>LDL, mg/dl</td>
<td>101±27</td>
<td>105±27</td>
<td>0.592</td>
</tr>
<tr>
<td>HDL, mg/dl</td>
<td>45±12</td>
<td>43±11</td>
<td>0.482</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD.

Table 2. Patient clinical characteristics and OSAS symptoms

<table>
<thead>
<tr>
<th>Clinical characteristic</th>
<th>Non-OSAS (AHI &lt;5; n = 31)</th>
<th>OSAS (AHI ≥5; n = 42)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>12 (38.8)</td>
<td>25 (59.5)</td>
<td>0.08</td>
</tr>
<tr>
<td>Female</td>
<td>19 (61.2)</td>
<td>17 (40.5)</td>
<td></td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td>2 (6.5)</td>
<td>4 (9.5)</td>
<td>1.00</td>
</tr>
<tr>
<td>Comorbid disease</td>
<td>17 (54.8)</td>
<td>27 (64.3)</td>
<td>0.473</td>
</tr>
<tr>
<td>Snoring¹</td>
<td>22 (71.0)</td>
<td>41 (97.6)</td>
<td>0.001</td>
</tr>
<tr>
<td>Witnessed apnea¹</td>
<td>3 (9.7)</td>
<td>29 (69.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Daytime sleepiness¹</td>
<td>4 (12.9)</td>
<td>23 (54.8)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Symptoms

| No major symptom         | 9 (29.0)                  | –                      |           |
| 1 major symptom          | 17 (54.8)                 | 8 (19.0)               | <0.001    |
| 2 major symptoms         | 3 (9.7)                   | 17 (40.5)              |           |
| 3 major symptoms         | 2 (6.5)                   | 17 (40.5)              |           |

Data are presented as n (%). ¹ Major symptoms of OSAS.

Table 3. Frequency of APOE genotypes and alleles in all cases

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Non-OSAS (n = 73)</th>
<th>OSAS (n = 42)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>E3/E3</td>
<td>55 (75.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E3/E4</td>
<td>7 (9.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2/E3</td>
<td>9 (12.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2/E4</td>
<td>2 (2.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2/E2</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E4/E4</td>
<td>–</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Allele</th>
<th>Non-OSAS (n = 73)</th>
<th>OSAS (n = 42)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>E3</td>
<td>71 (97.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E4</td>
<td>9 (12.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td>11 (15.1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as n (%).
The individuals with APOE2 alleles (E2/E3, E2/E4) compared to the individuals with only an E3/E3 allele genotype had a 10-fold greater OSAS risk (OR = 10.3, 95% CI 1.24–86.61, p = 0.0308). The individuals with APOE4 alleles (E2/E4, E3/E4) compared to the individuals with no APOE4 alleles (E2/E3, E3/E3) had a high but insignificant risk for OSAS (OR = 2.9, 95% CI 0.55–15.05, p = 0.286). The individuals with APOE4 (E2/E4, E3/E4) compared to APOE3 alleles (E3/E3) had an increased but insignificant risk for OSAS (OR = 3.62, 95% CI 0.96–19.05, p = 0.127). The individuals with positive compared to negative APOE2 alleles had significantly higher total cholesterol levels (182 ± 30 vs. 204 ± 28 mg/dl, respectively) but not triglyceride levels. No relationship was present between APOE2 alleles and LDL (low-density lipoprotein) and HDL (high-density lipoprotein). There was also no relationship between APOE3 and E4 and any type of lipids.

**Discussion**

There is growing evidence that genetic factors and their interaction with environmental exposures influence the development of OSAS. Well-established ethnic differences in the prevalence of OSAS and clinical observations suggest a genetic basis for OSAS. The evidence from family-based and twin studies, as well as the association of specific hereditary craniofacial disorders and the Prader-Willi syndrome with OSAS also indicate that the disease may have a genetic basis. However, most cases of OSAS do not exhibit classical Mendelian patterns of inheritance, suggesting a multigene pathogenesis, where many common variants with small or moderate genetic effects determine disease heritability. Phenotypes associated with OSAS, such as central obesity and ‘obstructive’ craniofacial morphometry also have a complex genetic basis. To date, approximately 30 candidate gene association studies have been performed in OSAS using various definitions of the syndrome in different populations. Apart from the association of the 308A single nucleotide polymorphism of tumor necrosis factor-α with OSAS, there has been no consistent replication of the findings of any of the studies. Unfortunately, most are not immune to the criticism of being underpowered, poorly controlled and poorly phenotyped [6, 24].

In the present study, APOE2 alleles compared to non-APOE2 alleles were shown to be significantly higher risk factors for OSAS. Moreover, APOE4 alleles were thought to have an increased risk for OSAS. We have found significantly higher total cholesterol levels in individuals with positive APOE2 alleles in contrast to an inverse relation in some other studies [9]. The E4 allele is associated with elevations in total cholesterol and with the risk of atherosclerotic diseases, while the E2 allele has the opposite effect [25, 26].

There are fewer studies regarding the APOE2 and OSAS association. Larkin et al. [27] reported an association-based analysis in the larger sample of 1,211 individuals, and observed a higher prevalence of sleep apnea among individuals with the APOE2 allele. In contrast, Kalra et al. [28] reported that E2 status is not associated with OSAS. However, a limitation of their study was that they lacked PSG data on their control group. In another study, Saarelainen et al. [29] reported that the distribution of APOE alleles, including APOE2 and genotypes, showed no difference between OSAS and controls. Cosenzino et al. [11] did not show any association between E2 status and OSAS. Another important point to consider with these three studies [11, 28, 29] is the difference between case definitions based on PSG evidence of AHI >1, AHI ≥5 or AHI ≥15, which in our study was AHI ≥5. Moreover, the control groups of the three studies were obviously different from the control group of the current study, which was proven to be non-OSAS by PSG. There-
fore, it is possible that a small proportion of their controls might have had OSAS. In a recent meta-analysis, 31 population-based studies, including the Kalra et al. [28], Saarelainen et al. [29] and Cosentino et al. [11] studies, were analyzed regarding APOE2 (3 studies, 506 cases/941 controls) and APOE4 (3 studies, 560 cases/1,048 controls). The E4 and E2 alleles were found not to be associated with OSAS [24].

In the present study, patients with APOE4 alleles had an almost significant risk for OSAS. After adjustment for age, sex and BMI, Gottlieb et al. [16] found that the presence of any APOE4 allele was associated with an increased risk of OSAS, particularly in individuals under the age of 65 years. A cohort study from Stanford University including 791 middle-aged adults (32–68 years) showed that the probability of moderate-to-severe sleep-disordered breathing was significantly higher in participants with E4, independent of age, sex, BMI and ethnicity. These effects increased with the number of APOE4 alleles carried [30]. In the current study, E4 and E2 alleles were not detected as risk factors, possibly due to the relatively low number of participants. Gozal et al. [31] reported that the APOE4 allele is more frequent in children with obstructive sleep apnea and particularly in those who develop neurocognitive deficits, suggesting that the APOE4 allele is associated with not only increased odds of having sleep-disordered breathing, but also with an increased risk for neurocognitive dysfunction. A study from France showed a potential interaction between sleep disorder breathing (SDB) and the APOE genotype, and suggested that the E4 allele might be a genetic risk factor for SDB (E4 allele frequency was higher in patients with moderate-to-severe SDB vs. the general population) and/or consequently a deleterious factor for this pathology (increased AHI in E4-positive vs. E4-negative patients). They speculated that depression might be only one of the clinical consequences of SDB [32]. Foley et al. [33] examined this association among 718 Japanese-American men aged 79–97 years who were evaluated for SDB and reported a weaker relationship observed in elderly adults compared with middle-aged adults. After adjusting for age, BMI, smoking and use of antihypertensive medications, they found no association between APOE4 and AHI greater than 15. Nevertheless, in a meta-analysis and a review, they reported that the existing evidence pointed towards an overall lack of association between possession of the APOE4 allele and risk of OSAS [6, 34]. The hypothesis that the APOE4 allele may be causally associated with OSAS cannot be supported by published literature. Also, any association seemed to decrease with increasing age.

In conclusion, the individuals with APOE2 alleles (E2/E3, E2/E4) compared to APOE3 alleles (normal variant APOE genotype) seemed to have a significantly higher risk factor for OSAS. The major limitation of this study was the low number of subjects. Nevertheless, the analysis of APOE genotypes can predict OSAS patients among a high-risk population. Finally, it is important to note that no genetic study, be it linkage or association based, can prove causation of a disease. Causation can only be proven when the pathologic function of the gene is demonstrated.

Financial Disclosure and Conflicts of Interest

All authors indicate that they have no financial relationship with the organization that sponsored the research. The authors declare that they have no conflict of interest.

References


