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Apolipoprotein E polymorphisms and sleep quality in Obstructive Sleep Apnea Syndrome

R. Pellegrino, D.R. Mazzotti, C. Guindalini *, R. Santos-Silva, L.R.A. Bittencourt, S. Tufik

Departamento de Psicobiologia, Universidade Federal de São Paulo, São Paulo-SP, Brazil

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ABSTRACT

Background: The purpose of this study was to evaluate the influence of polymorphism on sleep parameters of Obstructive Sleep Apnea Syndrome (OSAS) patients.
Methods: Patients were genotyped after a full-night polysomnography using the large Epidemiologic Sleep Study of São Paulo population-based sample.
Results: Individuals who carry the *APOE* ε2 allele showed longer sleep latency, lower sleep efficiency and higher numbers of arousals/hour, when compared to ε3 allele homozygous and carriers of ε4 allele ($p<0.05$). These findings remained significant even after correction for potential confounders, such as sex, age and African genetic ancestry.
Conclusion: The *APOE* polymorphisms may modulate the effects of intermittent hypoxia and sleep fragmentation in the sleep architecture of OSAS patients, and that the presence of the ε2 allele may serve as a biological marker for the identification of a subgroup of patients who are more likely to suffer with OSAS detrimental effects on sleep, impacting not only the daily functioning, but also their quality of life.

1. Introduction

The majority of sleep disorders are known to be a result of a complex interaction between environmental factors and individual genetic susceptibility. Obstructive Sleep Apnea Syndrome (OSAS) is characterized by repeated episodes of airflow reduction (hypopnea) or cessation (apneas) due to upper airway obstruction during sleep [1]. These events often generate hypoxemia and alterations in sleep architecture, such as frequent arousals (sleep fragmentation), reduced rapid eye movement (REM) sleep and slow wave sleep (stages 3 and 4), as well as consequent daytime somnolence [1]. Since the identification of a candidate region on chromosome 19, including the Apolipoprotein E (*APOE*) gene, a number of studies have evaluated possible associations between polymorphisms in this gene and the diagnosis of OSAS [2–10].

Human *APOE* is an apolipoprotein known to act directly in cholesterol transport and plasma lipoprotein metabolism [11]. Studies in mice have demonstrated that, aside from its role on lipid metabolism, *APOE* likely has other cellular activities, such as AKT-transduction signaling related to neuronal protection and survival, as well as the maintenance of the blood–brain barrier and differential response to injury by neurotrophic factors in the brain [12]. The most commonly studied *APOE* gene polymorphisms are characterized by amino acid substitutions at positions 112 (rs7412) and 158 (rs429358) of the peptide chain, where the most frequent alleles are ε2 (cysteine-112/cysteine-158—E2 isoform), ε3 (cysteine-112/arginine-158—E3 isoform), and ε4 (arginine-112/arginine-158—E4 isoform). These two substitutions can generate six genotypes of *APOE*: 3 homozygous (ε2ε2, ε3ε3, and ε4ε4) and 3 heterozygous (ε2ε3, ε3ε4, and ε2ε4) [13].

Links between sleep apnea, *APOE*, and aging disorders related to mental commitment observed in some dementia patients have been demonstrated. A more possible explanation of assumed mechanistic link between sleep apnea and aging effects can be related to dementia and its potential alterations in hemodynamic property of sleep disordered breathing on the cerebral circulation [2]. Genetic association studies evaluating OSAS phenotype and *APOE* polymorphisms have produced conflicting results [2,4–10,14], with 2 studies showing a higher risk of OSA in individuals carrying the *APOE* ε4 allele, one study reporting a higher risk of OSA in the presence of the *APOE* ε2 allele, and a total of five studies demonstrating no association between OSA and the *APOE* alleles. A recent meta-analysis, including the eight studies mentioned above has suggested that it is unlikely that the *APOE* polymorphisms influence the risk of OSAS itself, since the results do not support the previous associations between ε4 allele and OSAS [15]. However, the study highlighted promising evidence from previous studies that these polymorphisms, instead of conferring risk, may actually be involved in the modulation of the adverse consequences of sleep-disordered breathing, as demonstrated by studies in human and animal models, showing an influence of the *APOE* gene in neurocognitive dysfunction, brain injury, oxidative stress and inflammatory markers in response to intermittent hypoxia [7,12].

* Corresponding author at: Rua Napoleão de Barros, 925, Vila Clementino, SP—zip code 04024-002, São Paulo, Brazil. Tel.: +55 11 21490155; fax: +55 11 55725092.
E-mail address: camilascg@gmail.com (C. Guindalini).

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As a consequence of the respiratory events and the frequent arousals, in comparison with individuals without OSAS, patients with sleep disorder breathing show an overall increase of sleep stages 1 and 2, and an important decrease in stages 3, 4, REM sleep and total sleep time, which directly influences sleep efficiency and is thought to be the causes of excessive daytime sleepiness and reduced daytime functioning [16]. However, the magnitude of the impact of OSAS on sleep parameters is highly variable among patients, suggesting that genetic factors may play a role modulating the way individuals cope with the disease consequences. In this sense, we sought to evaluate if *APOE* polymorphism alleles may exert an effect on the objective sleep quality in patients with OSAS, as measured by full night polysomnography (PSG), in a large population-based sample from Sao Paulo, Brazil.

2. Methods

2.1. Studied population

APOE genotypes were examined in individuals participating in the São Paulo Epidemiologic Sleep Study, a population-based survey, in which 1042 subjects underwent PSG and were investigated through sleep questionnaires and blood sample measurements [17]. To obtain a representative sample of the inhabitants of Sao Paulo, we used a probabilistic three-stage cluster sampling technique and a sample size defined to allow for prevalence estimates with 3% precision. Pregnant and lactating women, people with physical or mental impairments that prevent self-care, individuals below 20 or over 80 years old, and people who work every night were not included in the household drawing. More details regarding sampling procedures can be found in [17]. The study protocol was approved by the Ethics Committee for Research of the Universidade Federal de São Paulo (CEP 0593/06) and registered with ClinicalTrials.gov (number: NCT00596713; name: Epidemiology of sleep disturbances among adult population of the Sao Paulo City). Informed consent forms were obtained and signed for all participants.

2.2. Polysomnography, OSAS assessment and blood samples collection

Individuals were subjected to one full-night PSG at the sleep laboratory and sleep stages were visually scored by four trained technicians according to standardized criteria for investigating sleep [18]. Diagnosis of OSAS was performed according to the International Classification of Sleep Disorders (ICSD-2) [19]. Individuals with OSAS were considered positive when their Apnea-hypopnoea index (AHI) was between 5 and 14.9 together with at least one of the following complaints: loud snoring, daytime sleepiness, fatigue, and breathing interruptions observed during sleep. Subjects with an AHI equal to or higher than 15 were also considered having OSAS, regardless of whether they had any of the aforementioned complaints.

In the day after the PSG, blood samples were collected for lipid serum levels measurements (total cholesterol, LDL, HDL, VLDL-cholesterol and triglycerides) and DNA extraction for polymorphism genotyping. A detailed version with further information about the methodology has been previously published elsewhere [17].

2.3. APOE and ancestry-informative markers genotyping

DNA was extracted from peripheral whole blood using a standard protocol [20]. The genotyping of all single nucleotide polymorphisms (SNPs) selected for this study was performed using allele-specific polymerase chain reaction with a molecular beacons assay under contract from Prevention Genetics (Marshfield, WI; http://www.preventiongenetics.com). The *APOE* polymorphism was characterized by the genotyping of two SNPs within *APOE* gene (rs7412 and rs429358) and the population stratification in the sample was estimated using a set of 31 ancestry-informative markers from Africa, Europe, and Native America. The number of ancestral populations (K) in the sample and individual admixture proportions was estimated using the Bayesian Markov Chain-Monte Carlo method implemented in the program Structure 2.1[21–23].

2.4. Statistical analysis

Hardy–Weinberg equilibrium was tested by calculating a χ^2 with 1 degree of freedom for each polymorphism that composes *APOE* polymorphism. We grouped individuals regarding their *APOE* genotypes as follows: ε2 allele carriers (ε2ε2 and ε2ε3 genotypes), ε3 homozygous (ε3ε3 genotype) and ε4 allele carriers (ε3ε4 and ε4ε4 genotypes), in order to verify the association regarding the presence of at least 1 polymorphic allele (ε2 or ε4). This approach was also adopted to increase the power to detect associations, once it reduces the number of tested categories and increases the number of observations per category. The sleep parameters measured by PSG were compared among the groups using Kruskal–Wallis and Mann–Whitney tests. In addition, a multivariate general linear model was used to correct for potential confounders. Spearman correlation analyses were performed between lipid levels and sleep parameters as well. Statistical analyses were performed using SPSS 18.0 (Chicago, IL, USA). Results are shown as mean ± standard deviation, and a p value of 0.05 was established as statistically significant.

3. Results

A total of 293 OSAS patients had valid *APOE* genotyping. *APOE* genotypes were in Hardy–Weinberg equilibrium ($p>0.05$), the genotype frequencies were 2.0% for ε2ε2 ($N=6$), 7.5% for ε2ε3 ($N=22$), 2.7% for ε2ε4 ($N=8$), 69.6% for ε3ε3 ($N=204$), 16.7% for ε3ε4 ($N=49$) and 1.4% for ε4ε4 ($N=4$), and the general allele frequencies were 7.2% for ε2, 81.7% for ε3, and 11.1% for ε4 allele. As ε2 and ε4 alleles are described to have opposite effects, the ε2ε4 genotype was excluded from analyses, and the final sample consisted of 285 individuals. This final sample was 56.8% male, with a mean age of 50.52 ± 13.80 years. More detailed information about socio-economic status, gender, age, body mass index and OSAS can be found elsewhere [1].

Concerning the lipid serum levels, carriers of the ε2 allele showed decreased LDL-cholesterol levels when compared to ε3 homozygous (mean difference = −13.3 mg/dL; $p=0.028$) and ε4 carriers (mean difference = −26 mg/dL; $p=0.003$). In addition, ε3 homozygous showed decreased LDL-cholesterol levels when compared to ε4 carriers (mean difference = −12.6 mg/dL; $p=0.025$).

When objective sleep parameters were compared among genotype groups, it was demonstrated that the ε2 allele carriers showed longer sleep latency in minutes (29.6 ± 25.0 vs. 16.5 ± 22.9 and 13.7 ± 13.4 for ε3 homozygous [$p=0.001$] and ε4 allele carriers [$p=0.001$], respectively), a lower percentage of sleep efficiency (70.3 ± 16.9 vs. 79.7 ± 12.5 [$p=0.002$] and 80.2 ± 11.2 [$p=0.006$]) and a higher index of arousals per hour (29.8 ± 16.0 vs. 21.2 ± 12.5 [$p=0.003$] and 22.3 ± 16.1 [$p=0.011$]) when compared to ε3 homozygous and ε4 carriers, respectively. Furthermore, ε2 allele carriers also spent significantly more time awake in minutes (98.9 ± 56.1), showed a higher percentage of stage 1 (6.0 ± 3.1) and a lower percentage of stages 3 + 4 (17.15 ± 8.4) when compared to ε3 homozygous (67.9 ± 45.2 [$p=0.002$]; 4.9 ± 3.3[$p=0.027$], 21.3 ± 8.6 [$p=0.016$], respectively). In addition, ε4 carriers spent more time in minutes awake than ε3 homozygous (71.9 ± 46.6 vs. 67.9 ± 45.2, respectively [$p=0.019$]). To account for multiple testing, a Bonferroni correction for pairwise comparison was applied, and the significance level was divided by three independent tests (ε2 vs. ε3; ε2 vs. ε4 and ε3 vs. ε4), resulting in $\alpha=0.017$. In this sense, only the comparison of percentage of stage 1 between ε2 and ε3 allele carriers ($p=0.027$), as well as the comparison of time awake between ε2 and ε4 allele carriers

Table 1
Sleep parameters obtained after polysomnographic recordings for Obstructive Sleep Apnea Syndrome patients among the three *APOE* genotype groups.

Variables	*APOE* alleles groups									p*
	ε2			ε3			ε4			
	N	Mean	SD	N	Mean	SD	N	Mean	SD	
Sleep latency	28	29.59	25.00	204	16.53	22.88	53	13.68	13.44	0.002[a,b]
REM latency	28	123.48	78.15	204	106.14	58.22	53	99.69	51.70	NS
Total sleep time	28	309.54	87.39	204	329.64	73.14	53	341.23	59.83	NS
Sleep efficiency	28	70.28	16.93	204	79.75	12.50	53	80.24	11.20	0.007[a,b]
% Stage 1	28	6.05	3.06	204	4.88	3.29	53	5.42	3.34	0.049[a]
% Stage 2	28	58.72	10.50	204	55.58	9.80	53	56.45	9.18	NS
% Stages 3 and 4	28	17.15	8.42	204	21.26	8.27	53	19.45	8.03	0.039[a]
% REM Sleep	28	18.08	8.98	204	18.28	6.50	53	18.68	5.33	NS
Time awake	28	98.95	56.08	204	67.89	45.25	53	71.87	46.60	0.008[a,b]
Arousals/h	28	29.85	16.02	204	21.17	12.49	53	22.32	16.15	0.011[a,b]

* Kruskal–Wallis test.
[a] $p<0.05$, ε2 × ε3 pairwise comparisons.
[b] $p<0.05$, ε2 × ε4 pairwise comparisons.

($p=0.019$) did not remain significant. Table 1 shows the results for all the measured sleep parameters and Fig. 1 depicts the statistically significant results in bar charts. No significant differences were found between ε3 homozygous and ε4 allele carriers concerning the other sleep parameters ($p>0.05$; Table 1). Interestingly, in an exploratory analysis, we have performed the same analyses in a sample of 580 individuals without OSAS and found no statistically significant difference in any of the sleep parameters among the genotypes (data not shown).

To verify whether potential confounders might influence the aforementioned associations, the *APOE* ε3 homozygous and ε4 allele carriers were grouped and tested against the ε2 allele carriers in a multivariate general linear model. Sleep latency, sleep efficiency and an index of arousals/hour were used as dependent variables and sex, age and African genetic ancestry were used as factors and covariates in the model. After correction, ε2 allele carriers still showed longer sleep latency (Mean difference = 13.06; 95%CI = 4.61 to 21.51; $p=0.003$), lower sleep efficiency (Mean difference = −8.51; 95% CI = −13.22 to −3.80; $p<0.001$) and a higher number of arousals per hour (Mean difference = 7.80; 95%CI = 2.52 to 13.08; $p=0.004$) than ε3 homozygous/ε4 allele carriers.

We also conducted correlation analyses between the lipid levels and the significant sleep parameters and, interestingly, we found that HDL-c levels were positively correlated with sleep latency (Spearman's $\rho=0.16$; $p=0.003$) and time awake (Spearman's $\rho=0.20$; $p<0.001$) as well as negatively correlated with sleep efficiency (Spearman's $\rho=-0.24$; $p<0.001$). Total cholesterol levels were also positively correlated with sleep latency (Spearman's $\rho=0.13$; $p=0.020$). Although all of these correlations were weak, we decided to verify the effect of HDL and total cholesterol levels on the associations between *APOE* genotype groups and the sleep parameters using general linear models. Regarding the effect of the genotype groups on sleep latency, the presence of HDL and total cholesterol as covariates in the model did not interfere the association with *APOE* genotypes ($p=0.005$). In addition, when both sleep efficiency and time awake were modeled with HDL as covariate, the effect of the genotype groups also remained significant ($p=0.001$ and $p=0.006$, respectively). These results suggest that the effects of *APOE* genotype groups on studied sleep parameters are independent of the correlation between lipid levels and these parameters.

4. Discussion

Patients who carry the ε2 allele demonstrated longer sleep latency, lower sleep efficiency and a higher index of arousals per hour, when compared to ε3 homozygous and individuals carrying the ε4 allele. Moreover, ε2 allele carriers also spent significantly more time awake and showed a higher percentage of stage 1 and a low percentage of stages 3 + 4 compared to ε3 homozygous. The differences in sleep latency and efficiency, as well as in the index of arousals per hour observed in *APOE* ε2 allele carriers remained significant, even after correction for potential confounders such as age, sex and African ancestry and correlated lipid levels. In addition, we verified the well-known association between ε2 allele and lower LDL-cholesterol, suggesting that this sample can estimate correctly the effects of *APOE* polymorphism on lipid levels measurements and that it can be representative for association studies regarding *APOE* polymorphism.

APOE, especially the polymorphisms contained in the three common alleles ε2, ε3, and ε4, is one of the most thoroughly studied genetic polymorphisms in humans [24]. Previous results have demonstrated a higher risk of developing OSAS in individuals carrying the ε4 allele [6,8], as well as the ε2 allele [9]; however, the association between *APOE* alleles and this sleep disorder is not completely elucidated, with a series of negative findings. Agreeing with our results, recent approaches such as meta-analysis and knock-out mice studies indicated that, rather than increasing the vulnerability to OSAS themselves, this genetic polymorphism of *APOE* may actually be related to the modulation of the adverse effects of OSAS [12,14,25]. In this study, we evaluated the potential impact of *APOE* alleles on the objective sleep parameters, which are highly affected in individuals with OSAS, and are directly associated with the negative consequences of OSAS, such as excessive daytime somnolence and poorer cognitive performance. With the vast majority of the functional studies available to date evaluating the effect of the ε3 and ε4 alleles only, the mechanisms involved in the modulation of the ε2 allele's effects on sleep parameters of OSAS patients are currently unknown. Nevertheless, interestingly, although the ε4 allele has been commonly associated with various adverse health conditions, as well as a higher susceptibility to Alzheimer's and cardiovascular diseases [26–29], several negative effects of the ε2 allele have also been observed for a number of disorders such as inflammatory diseases, hyperlipoproteinemia type III, macular degeneration, tardive dyskinesia and colorectal cancer, suggesting a plausible mechanism for a context-dependent deleterious effect of the ε2 and ε4 alleles [29]. Agreeing with this hypothesis, we have demonstrated in a larger samples including individuals not diagnosed with OSAS that the association between APOE polymorphism and various sleep parameters was not observed, suggesting that the influence of this polymorphism is restricted to the hypoxia and/or sleep fragmentation context of OSAS phenotype. Moreover, sleep dysfunction and excessive daytime somnolence have long been recognized as common features in patients with Parkinson disease (PD) [30]. Although still debatable, results from a meta-analysis evaluating twenty-two eligible studies suggested that the ε2 allele, and not ε4, is associated with an increased risk of developing PD [31].

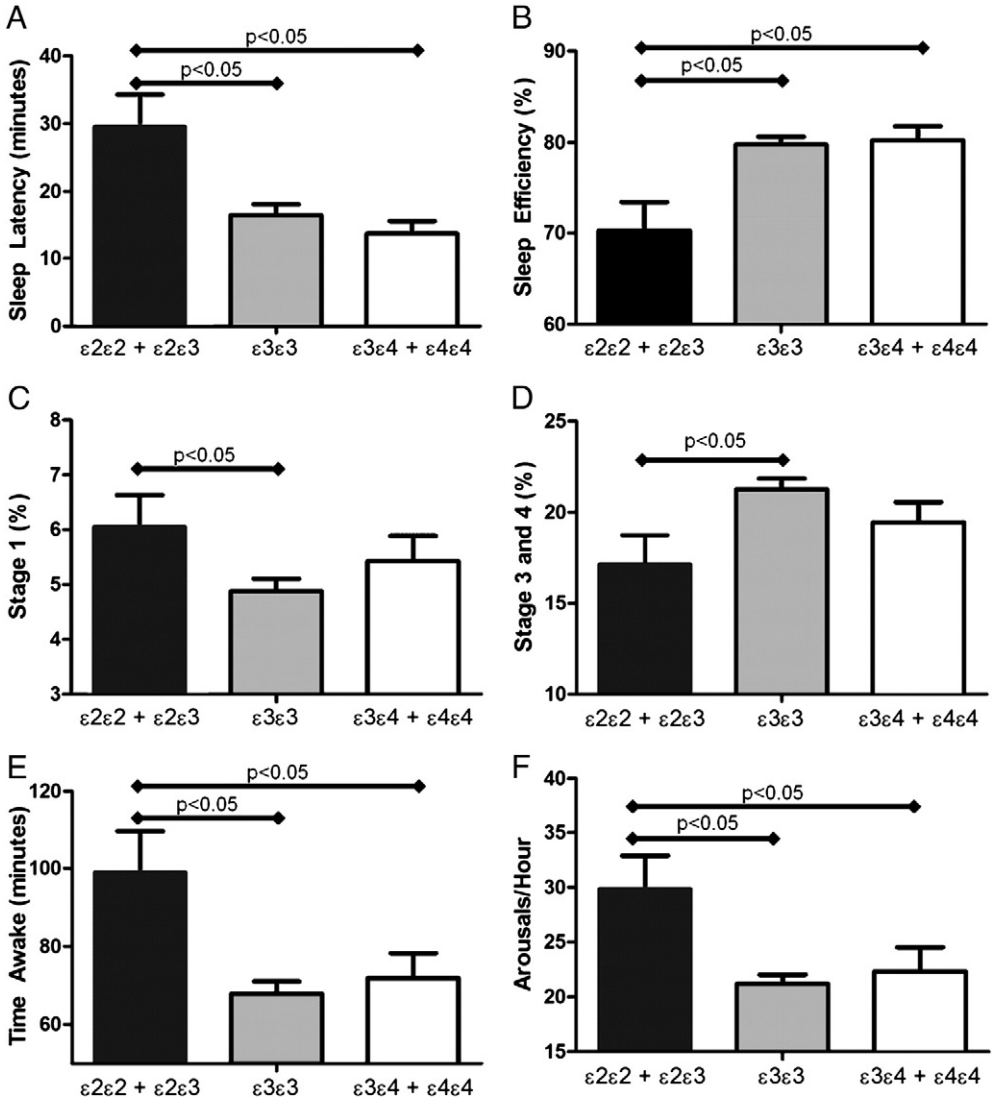

Fig. 1. Significantly associated sleep parameters between *APOE* genotypes in the Obstructive Sleep Apnea Syndrome patients. Error Bars represent Standard Error.

Taken together, these results may suggest that not only the *APOE* ε4 allele, but also the ε2 may indeed exert negative effects on disease-related phenotypes. Initially recognized as a key determinant in lipoprotein metabolism, functional studies have shown that, consistent with their protective roles in AD, the E2 and E3 isoforms of the APOE molecule are also associated with facilitation of neurite outgrowth and apoptosis inhibition, more efficiently than the APOE4 [32]. Nevertheless, considering the wide expression of APOE across the body and the variability of the structural and biophysical properties among the three isoforms [32,33], it is possible that other neuronal homeostasis or physiological functions associated with the APOE2 variant may hold the clue for understanding its relationship with reduced sleep quality in patients with OSAS. In this sense, further functional studies are necessary before we can confirm the effect of the ε2 allele on sleep parameters of OSAS patients. In addition, the apparent lack of agreement between the different studies evaluating the association between *APOE* alleles/genotypes and OSAS is consistent with the idea, already mentioned by Larkin et al. [9], that other OSAS-related locus near *APOE* gene and in close linkage disequilibrium with it exists and it is actually responsible for the linkage peak on chromosome 19, which first gave rise to the speculation that the *APOE* gene could be involved in the risk of OSAS.

In summary, our results demonstrate an association between the ε2 allele and reduced sleep quality in patients with OSAS, suggesting that polymorphisms of *APOE* and its regulatory region may modulate the deleterious effects of intermittent hypoxia and sleep fragmentation in the sleep architecture of OSAS patients. Additionally, the findings of the present study support the concept that genetic factors may help explain the inter-individual phenotypic variations and serve as a useful biological marker for the identification of subgroups of individuals suffering with OSAS. Clearly, more controlled studies, involving paired individuals with all genotype groups should be addressed to elucidate the true effect of *APOE* polymorphism and OSAS on sleep measurements and the replication of these findings in other populations and study designs is essential for establishing the function of *APOE* in the pathophysiology of OSAS.

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