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Original Article

Evidence for a contribution of the APOE (but not the ACE) gene to the sleep profile of non-demented elderly adults

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Abstract: This study aims to investigate alleles of the human apolipoprotein E (APOE) and of the angiotensin-converting enzyme (ACE) genes as risk factors for poor quality of sleep in elderly individuals with no major cognitive decline. This cross-sectional, analytical study was conducted with 163 participants aged 75 years in average and 85% female. Sociodemographic, anthropometric and clinical data were gathered, and sleep quality was assessed using the Pittsburgh Sleep Quality Index (PSQI) and the Epworth scale, with patient followed for years prior to these evaluations to rule out onset of major mental disorders. Genotyping of classic polymorphic sites for the ApoE (rs429358 and rs7412) and the ACE (rs4646994) genes used peripheral DNA. A total of 63% of the subjects reported poor quality of sleep assessed by the PSQI whereas 54 (33%) reported daytime sleepiness through the Epworth scale. A significant correlation was observed between APOE and PSQI, with a greater frequency of the poor nighttime sleep quality phenotype among ε2 carriers, whereas no correlation was found among any of the sleep scores and the ACE genotypes. Thus, we suggest a correlation between APOE alleles and scale-assessed sleep quality scores in older adults, with no implications for ACE alleles, in a context devoid of cognitive impairment.

Keywords: Apolipoprotein, genotyping, sleep, older adults, cognition disorders

Introduction

Good quality sleep is important in sustaining the individual's physical functioning and psychiatric well-being [1]. However, one third of the population is affected by sleep complaints, rendering major social, medical, and/or economic impact to subjects, families and society [2]. Sleep problems in old age are prevalent and are known to associate with physical and psychological factors. Survey revealed that over 50% of all adults aged 55 to 84 years-old had sleep complaints such as trouble in falling asleep or waking up repeatedly at night [1].

Sleep is influenced by genetic and environmental factors [3]. Partinen et al. [4], found high estimates of heritability for sleep length and sleep quality in twin adults ($h^2 = 0.44$). Also, the overall sleep pattern (total number of hours slept) of monozygotic twins living apart compared to that of monozygotic twins living together were found to be correlated ($r = 0.53$ and $r = 0.49$, respectively), implying that genetic elements pose an important contribution to determine total sleep time [5].

Understanding the genetic basis of sleep disorders is important because it leads to insights about its pathophysiology, and may lead to new diagnostic tests and more importantly to novel, personalized therapies for patients with sleep disorders [6]. Kadotani and colleagues found statistical association between the ε4 allele of the apolipoprotein E gene (APOE) and sleep apnea in a sample aged 32 to 68 years [7]. However, Foley and colleagues examined this association among Japanese-American men aged 79 to 97 years and, after adjusting for age, body mass index, smoking and use of antihypertensive medications, found no association between ε4 and apnea-hypopneia indexes greater than 15 (odds ratio [OR], 0.77; 95% confidence interval [CI], 0.52-1.14) [8]. A polymor-

phism in the angiotensin-converting enzyme was also reported to be associated with moderate obstructive sleep apnea syndrome, especially in hypertensive patients [9].

APOE is located in chromosome 19, and produces different protein isoforms based on three existent polymorphic variants (ε2, ε3 and ε4). Their frequencies vary substantially around the world, with ε3 as the most common form in almost every population, being considered the 'wild-type' allele [10]. The ε4 variant is the largest known genetic risk factor for the late-onset, sporadic form of the Alzheimer disease [11] and is related with a worsened prognosis in patients with multiple sclerosis and Parkinson's Disease [12].

Regarding the ACE gene, Rigat and others described its main variation, consisting of an insertion/deletion polymorphism encompassing 287 base pairs within a noncoding region [13]. This alteration yields three genotypes (II, DD, ID), and the D allele is believed to produce 60% higher activity of the enzyme in the serum [13, 14]. Evidence from several animal and human studies strongly supports involvement of this multi-enzyme system in the pathogenesis of vascular disorders. Subjects with the DD genotype had increased risk for myocardial infarction as well as for left ventricle hypertrophy, post-infarction remodeling, and idiopathic arterial hypertension [15]. Despite the overwhelming amount of information regarding the contribution of the APOE and ACE genes to classic disorders as dementia and infarction, respectively, proper description of their association with less common clinical traits remain elusive and should be addressed.

All in all, finding ways to minimize sleep disorders in the elderly population has consistently been associated with improvement in quality of life, regardless of health status and biological background, and is an important public health action [16]. In this study, we aimed to investigate the APOE and ACE alleles as genetic risk factors for poor quality of sleep in non-demented elderly individuals devoid of prior cognitive decline.

Materials and methods

Study design and patients

This was a cross-sectional, analytical study with non-demented elderly individuals who attended from August 2015 to July 2016 to the cohort work know as Prognosis and Therapeutics in Geriatrics (ProTeGer) in Brasília, Brazil. The subjects were admitted at two general geriatric outpatient clinics in the Brazilian Federal District (The Geriatric Center of the University of Brasilia and the Geriatrics Service of Catholic University of Brasilia).

Medical records were initially used only as source of preliminary information and initial selection, with due consultations performed as described latter to assure eligibility. But at this initial admission, medical records were sought for a validated Brazilian Portuguese version of the Mini-Mental State Examination (MMSE), with cut-off scores set at 18 and 26 points for individuals with ≤ 7 years and ≥ 8 years of formal education, respectively [17]. Inclusion criteria were being aged 60 years or older and exhibiting no cognitive decline according to the MMSE. Exclusion criteria were medically registered sleep complaints or disorders associated with acute conditions (uncompensated illnesses and recent psychiatric episodes), severe agitation or unstable medication use (prescription changes within 8 weeks) between last visits.

Clinical procedures

Individuals were invited to participate in structured interviews to identify their sociodemographic and clinical characteristics. Data were collected on identity, age (years), gender (male/female), level of schooling (years), body mass index (BMI, kg/m^2), geriatric depression scale (EDG score), comorbidities (yes/no), and self-reported health condition (excellent/very good/good/bad/very bad), as well as on smoking (yes/no) and alcohol consumption (yes/no). Systemic arterial hypertension (SAH) presence (yes/no) was defined as repeatedly elevated blood pressure ≥ 140 (systolic) and/or ≥ 90 (diastolic) mmHg while type-2 diabetes occurrence (yes/no) was defined by fasting glycated hemoglobin ≥ 6.5% and/or use of antidiabetic drugs, considering data from medical records. Current use of drug classes as hypnotics, antidepressants or other psychoactive agents that could interfere with sleep was also investigated and recorded (yes/no). During the interviews, disorders that could interfere with a participant's overall state of health (e.g. cancer, epilepsy, immobility, and stroke), such as important sensory (visual and/or auditory) impair-

ment, were identified by the clinical team and considered as further exclusion criteria.

Because all participants had been recruited for previous clinical and molecular studies [18], with patient's admission in the geriatric services having occurred up to 2012, cognitively intact patients at baseline could be monitored to exclude the possibility of cognitive decline over time by means of subsequent evaluations. Therefore, clinical reassessments were performed at least once for each patient between 48 and 78 months after the initial cognitive evaluation on admission at the health center, with only patients showing no clinically important decline during the period being enrolled for evaluations concerning their sleep profile.

The instrument used for nighttime sleep assessment was the Pittsburgh Sleep Quality Index (PSQI), a clinically useful instrument to measure the quality and pattern of sleep in the older adult [19]. The PSQI global score is calculated by summing scores of seven subscales, resulting in a range from 0 to 21. A global sum of 5 or greater indicates a poor quality of sleep. On its turn, the Epworth scale (ESS) was used to assess daytime sleepiness [20]. Ranging from 0 to 24, global scores of 10 or higher indicate daytime excessive somnolence. The applications of the questionnaires occurred individually during a regular clinical visit, being filled out with help of the researchers who explained the purpose of each question seeking to facilitate the patient's understanding without interfering with the answers.

This study was approved by the institutional ethics committee and conducted according to the Helsinki Declaration. Participation was voluntary, and written informed consent was obtained from each participant.

Genotyping procedures

The total genomic DNA of each participant was collected using routine laboratory procedures. Genotyping the common human APOE alleles were established according to a modified version of a method developed elsewhere [21] to determine the rs429358 and rs7412 variations by means of a multiplexed polymerase chain-reaction (PCR) using primers sense (5'-ATGCCGATGACCTGCAGAATR-3') and antisense (5'-CGCGGACATGGAGGACGTTR-3') designed so that the 3'-most base recognized either A or G corresponding to Arg or Cys at positions 112 or 158, respectively. Genotypes were identified based on the patterns of 588 and 451 base pairs (bp) amplicons generated. On its turn, the insertion (I)/deletion (D) polymorphism of the human ACE gene (rs4646994) was determined by inspection of the electrophoretic profile of PCR products, as described elsewhere and performed with modifications [22]. The 490 bp (I allele) and the 190 bp (D allele) products were amplified using primers sense (5'-CTGCAGACCACTCCCATCCTTTCT-3') and antisense (5'-GATGTGGCCATCACATTCGTCAGAT-3') flanking the polymorphic site. Confirmation of the DD genotypes was run using specific oligonucleotides (5'-TGGGACCACAGCGCCCGCCACTAC-3' and 5'-TCGCCAGCCCTCCCATGCCCATAA-3') to amplify an internal segment (335 bp) of the insertion sequence.

Each reaction tube contained 100 ng of DNA, 10 mmol/l of Tris-HCl pH 8.3, 75 mmol/l of KCl, 3.5 mmol/l of $MgCl_2$, 0,2 mmol/l of dNTPs, 20 pmol of each primer, 0.5 µg of purified chicken albumin and 1 U of Taq DNA polymerase (Phoneutria®, Minas Gerais, Brasil) in a final volume of 25 µl. After 1 min of hot start at 80°C and an initial denaturation for 2 min at 94°C, the amplifications were done for 30 cycles of 40 s at 94°C, 45 s at 64°C and 50 s at 72°C followed by a final 5 min extension at 72°C. Reactions were run to confirm the DD genotypes of the ACE gene by amplifying a fragment of the insertion sequence. All PCR products were separated by electrophoresis on 2% agarose gels containing ethidium bromide at 50 µg/ml, visualized by using CCD camera (Vilber Lourmat®, Eberhardzell, Deutschland), and examined using the gel analysis software enclosed (Photo Capt 1D) with confirmation by visual inspection comparing to a 50-bp molecular mass marker.

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences Version 17 (SPSS). Chi square test with Fisher's exact proportion and Spearman's correlations were used to study a possible correlation between variables. A p value of < 0.05 was considered significant. A second analysis using a controlled correlation test was performed inserting

Table 1. Anthropometric, clinical and metabolic variables of the sample (n = 163)

Variables	Value
Sociodemographic characteristics	
Female, n (%)	139 (85.2)
Age, years	75.3 ± 7.0
BMI, $kg.m^{-2}$	26.5 ± 4.7
Schooling, years	4.3 ± 3.7
Clinical conditions, n (%)	
T2DM	30 (18.5)
SAH	127 (78.4)
Smoking	26 (15.9)
Alcoholism	9 (5.5)
Medications, n (%)	
Tricyclic antidepressants	11 (6.8)
Non-tricyclic antidepressants	54 (33.3)
Anticonvulsant drugs	13 (8.0)
Benzodiazepines	10 (6.1)
Hypnotic drugs	10 (6.2)
Instrument, n (%)	
PSQI (poor sleep quality)	103 (63.1)
Epworth (excessive diurnal somnolence)	54 (33.1)
MMSE, points	24 ± 3.7
Geriatric depression scale (score ≥ 6)	45 (27.5)
Self-reported health condition	
Excellent/Very good/Good	12 (78.4)
Bad/Very bad	35 (21.3)
Genotypes, n (%)	
ECA	
DD	52 (31.9)
DI	85 (52.1)
II	26 (15.9)
APOE	
ε2 (ε2ε2, ε2ε3)	14 (8.5)
ε3 (ε3ε3)	121 (74.2)
ε4 (ε3ε4, ε4ε4)	28 (17.1)

Data are expressed as average ± standard deviation (SD) for continuous parameters or absolute count (with relative frequencies in parenthesis) for categorical features. BMI = body mass index; T2DM = type 2 diabetes mellitus; SAH = systemic arterial hypertension.

interfering variables into the model in order to verify if the original association is maintained.

Results

A total of 163 participants were included in this study, aged 75 years in average and 139 (85%) being female. Most volunteers reported schooling of 4 years and an overall good health condition (66%). Of all, 103 (63%) of the subjects reported poor quality of sleep by means of the PSQI whereas 54 (33%) reported daytime sleepiness through the Epworth scale. Homozygotes for ε3 were 74% of the sample (n = 121), and the genotypes were in perfect Hardy-Weinberg equilibrium (P > 0.05). Other baseline characteristics are reported in **Table 1**.

In descriptive terms purely, the sample was remarkably abundant of diabetic and hypertensive individuals, with only 30 (18.4%) devoid of either of these entities, denoting high prevalence of metabolic disorders as expected from the elderly of a developing country. But qui-square analyses showed no frequency variation of these conditions across genotypes. In line with a profile of multimorbidity, consumption of psychotropic agents also was unneglectable, with users of any of these drugs accounting for nearly half (n = 78; 47.8%) of our sample, and with one quarter (n = 20; 25.6%) of the users taking two or more active principles simultaneously. Again, analyses failed to reveal variation in consumption of these drugs across genotypic groups. Accordingly, neither the sociodemographic data (gender, age, schooling) nor the other self-reported or clinically assessed scores (depression, BMI, self-reported health, smoking or alcohol intake) deviated in frequency or mean values according to APOE or the ACE genotypes.

A significant correlation was observed between APOE and PSQI (**Table 2**), with a greater frequency of the poor nighttime sleep quality phenotype among ε2 carriers, whereas no correlation was found among these scores and ACE genotypes (**Table 3**). Also, no relationship was found between the APOE or the ACE genotypes with the Epworth assessed scores or with other clinical and sociodemographic variables determined in the sample. Correlation tests between ApoE and PSQI controlling for MEEM and EDG were run, and the association remained in place (r = -0.213; P = 0.007). The same was made with ApoE and Epworth, controlling for

Table 2. Distribution of subjects according to sleep profiles across genotypic groups of the ApoE gene, following to the Pearson chi-square test

Scale	Category	ApoE genotypes			*P**
		ε2	ε3	ε4	
Epworth	Excessive diurnal somnolence	5 (35.7)	41 (33.9)	8 (28.6)	0.845
PSQI	Poor nighttime sleep quality	12 (85.7)	78 (64.5)	13 (46.4)	0.038

Data expressed as absolute count (and proportion, in parenthesis) within genotype. *Chi squared test.

Table 3. Distribution of subjects according to sleep profiles across genotypic groups of the ACE gene, following to the Pearson chi-square test

Scale	Category	ACE genotypes			*P**
		DD	DI	II	
Epworth	Excessive diurnal somnolence	23 (44.2)	23 (27.1)	8 (30.8)	0.112
PSQI	Poor nighttime sleep quality	32 (61.5)	52 (61.2)	19 (73.1)	0.522

Data expressed as absolute count and proportion within genotype (in parenthesis). *Chi squared test.

MEEM, and an association remained non-existent (r = -0.035; P = 0.661).

Discussion

The present study did identify a correlation between classic genotypes of APOE and poor sleep quality at nighttime in elderly individuals clinically identified as having preserved cognitive functions.

Similarly to our report, Drogos et al. [16] conducted a study that investigated the association between sleep quality and the APOE gene in healthy older adults. Using in-home polissonography and actigraphy (to produce objective parameters of sleep) and the PSQI (for subjective evaluation), a significant relationship between the presence of the ε4 allele and objective sleep disturbances was found, but not with subjective sleep complaints. In our study, regardless of only subjective parameters being measured, the main risk factor for poor sleep quality relied on the APOE ε2 allele. This apparent inconsistency with the findings of Drogo and colleagues may derive from the fact that our study was conducted in a context devoid of cognitive decline, since our protocol excluded from analysis those that developed impairment over a long follow up. Another possible explanation relies on the weak relationship between subjective complaints of sleep (including PSQI scores) and objective measures of sleep, with perception measured through questionnaires reflecting distinct dimensions of those mapped through actigraphy and polysomnography [23].

In the literature, some studies relate the ε4 allele to the appearance of obstructive sleep apnea (OSA) in elderly patients and other respiratory events during sleep [24, 25]. However, a larger body of evidence aiming at measuring sleep problems in elderly sample point out contrariwise, showing that ε4 carriers present lower levels of snoring and of sleep apnea compared to non ε4 carriers. Uyrum & Balbay, for instance, studying a sample of 73 patients aged 51 years old in average found that the individuals with at least one APOE ε2 allele (ε2/ε3, ε2/ε4), compared to the individuals with no APOE ε2 alleles (ε3/ε3, ε3/ε4), had a 9.37-fold greater OSA risk [26]. In another report, individuals with APOE2 alleles (ε2/ε3, ε2/ε4) compared to the individuals with the ε3/ε3 genotype had a 10-fold greater OSA risk [10]. Lastly, Larkin *et al*. [27] reported a significant relationship between APOE ε2 and increased risk of OSA (P = 0.039). One meta-analysis evaluated the association between the APOE alleles and the risk of OSA and the results revealed that APOE profiles did not contribute to the phenotype [10].

All in all, genetic epidemiological studies have shown inconsistent and often nonreproductible findings regarding the association between APOE ε2/ε3/ε4 alleles and OSA susceptibility [28], and no conclusive association has yet been demonstrated between the APOE gene and sleep disturbance, sleep short of breath/awaking, sleep adequacy, or daytime somnolence [29]. A meta-analysis published by Thakre et al. [30] concluded that the hypothesis of a sound association between APOE alleles and

obstructive sleep apnea cannot either be sustained or ruled out based on literature published so far.

One report associating the APOE genotype and the risk for coronary artery disease concluded that effects of this gene polymorphism are plastic, highly depend on environmental and coexisting factors to determine actual risks [31]. Our findings might reflect such plasticity, and a better understanding of the role of this common genetic variation in the sleep profile may permit the identification of subgroups that are at increased risk to the development of sleep-deprivation disorders, especially by comparing diverse scenarios as groups more or less likely to develop late-onset dementia [16].

It is important to emphasize that no correlation between ACE genotype and excessive diurnal somnolence or poor nighttime sleep quality was observed in our settings. In line, a meta-analysis published by Lee et al. did not observe any correlation between ACE gene polymorphism and risk of OSAS development or disease severity, with no significant correlation between the presence of D allele and risk of OSA observed after stratification to ethnic origin or comorbidities, particularly arterial hypertension [32].

In summary, it is possible to notice in the literature that a correlation between the genotypes of APOE and sleep disorders is cogitated. Nonetheless, the authors could not elaborate any satisfying explanation for the intriguing phenomena reported herein apart from a possible exacerbation in obstructive sleep apnea prevalence among ε2 carriers. Therefore, we are prone to interpret our finding as limited evidence for a link that should be addressed with caution, and further investigated thoroughly.

This study has limitations. One is that specific sleep disorders (as short total sleep time, nighttime awakenings and long latency) were not determined. Moreover, we did not perform a power calculation before starting the study, and the small sample size could be considered a shortcoming. Nonetheless, the authors understand that the report poses a contribution to the literature by providing evidence in a context devoid of major mental disorders, since patients were followed over time prior to the analyses so to allow exclusion of those with important cognitive decline or psychiatric conditions. Moreover, clinical assessments took into account consumption of sleep-interfering psychoactive drugs, and since analyses failed to reveal unequal distribution of users/non-users across genotypes, the authors do not believe that the associative results here observed were pharmaceutically influenced.

Conclusion

In this study, we found correlation between the ε2 allele of APOE and a poor quality of sleep in older adults, and no association of ACE alleles with any of the scale-assessed sleep scores in a context devoid of cognitive impairment.

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Disclosure of conflict of interest

None.

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