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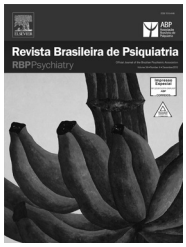

ORIGINAL ARTICLE

Homozygosity for the *APOE* E4 allele is solely associated with lower cognitive performance in Brazilian community-dwelling older adults – The Bambuí Study

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Abstract
Objective: To investigate if APOE E4 allelic status is associated with the cognitive functioning of elderly individuals. Methods: Participants (n = 1,408) from the Bambuí Cohort Study of Aging were selected based on the results from both variables (APOE genotype and MMSE score). Gender, age, education, marital status, skin color, GHQ score and biological measures were used as confounding factors for adjusting the logistic regression. *Results*: The population was in Hardy-Weinberg equilibrium, and the APOE E4 allele frequency was 13.4%. *APOE* E4 allele homozygosity conferred a superior odds ratio (OR) for cognitive impairment (OR = 3.1) compared to E4 allele heterozygosity (OR = 0.99) even when adjusted for age, sex, education, marital status, skin color, triglycerides, HDL, systolic pressure, and GHQ (OR = 2.9). No differences were observed between the other covariates. *Conclusions*: The APOE E4 allele was observed to have a dramatic effect on cognitive impairment, especially in homozygotes, which comprised approximately 2% of the population.

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DESCRITORES:
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Homozigose para o alelo E4 do gene APOE está associada à função cognitiva de idosos vivendo em comunidade – Projeto Bambuí

Resumo
Objetivo: Demonstrar que a presença do alelo *APOE* E4 está associada ao declínio cognitivo em idosos vivendo em comunidade. *Método*: Participaram do estudo 1408 residentes na cidade de Bambuí (MG) com 60 ou mais anos de idade. A variável dependente do estudo foi a função cognitiva, mensurada pelo Mini Exame do Estado Mental (MEEM). A variável de interesse do estudo foi o genótipo da apolipoproteína E (APOE). Para efeito de ajustamento na regressão logística foram consideradas como covariáveis o sexo, escolaridade, cor da pele, estado civil, sintomas depressivos, dosagem de triglicerídeos, HDL e pressão sistólica. *Resultados*: A frequência alélica do gene *APOE* (E4, E3, E2) mostrou distribuição em equilíbrio de Hardy-Weinberg. Foi detectada uma forte associação entre a presença do alelo E4 e o comprometimento cognitivo quando em homozigose (OR: 3,1; IC 95%: 1,39–6,99) mesmo após ajustamento por todas as potenciais variáveis de confusão (OR: 2,9; IC95%: 1,15–7,71). *Conclusões*: Os resultados mostraram que existe uma forte associação entre a presença do alelo E4 da APOE e a função cognitiva em idosos. Esta associação existiu somente entre indivíduos homozigotos (E4E4), indicando dependência da dose gênica no comprometimento cognitivo.

Introduction

Polymorphisms of the apoliprotein E gene (*APOE*) are one of the most important and well-replicated genetic risk factors for Alzheimer's disease (AD).[1] *ApoE* is a 299 amino acid plasma glycoprotein that plays a major role in lipid metabolism and is directly involved in amyloid plaque formation in the human brain. Two polymorphisms in the *APOE* coding region result in three alleles, *APOE* E2, *APOE* E3 (the most common allele) and *APOE* E4, and three major isoforms of the protein, each having different properties. Pooled results from association studies demonstrated that *APOE* E4 carriers have a 3- to 4-fold increased risk of developing AD, in contrast to the 10- to 12-fold increase that is observed for *APOE* E4 homozygotes.[2] There is also currently interest in the role of *APOE* E4 as a risk factor for cognitive performance in cognitively intact subjects.[3-5] However, the results of these studies are contradictory; some show an increased risk for cognitive decline in association with the allele status, and some show no association.[6,7] Brazilian studies have focused primarily on the relationship between the *APOE* gene and pathological cognitive decline (i.e., Alzheimer's disease) in individuals who were attended at health-service locations.[8-11] Only one previous community-based study has examined the relationship between *APOE* E4 and cognitive function. This study demonstrated a lack of association between the *APOE* E4 allele and cognitive impairment in cognitively intact, community-dwelling elderly individuals.[12]

The current study aims to investigate the relationship between the presence of the *APOE* E4 allele and cognitive performance in a large, community-based sample of Brazilian elderly individuals. The study will consider several potential confounding factors that were previously described to be related with cognitive impairment.

Methods

The Bambuí Cohort Study of Aging

The analysis presented herein was performed using data from the Bambuí Cohort Study of Aging[13] as a baseline. The Bambuí Cohort Study is a longitudinal, population-based study on the health and aging of participants aged at least 60 years from Bambuí, a town of 15,000 inhabitants in Minas Gerais State, Southeastern Brazil. A complete census was performed during November and December 1996 to choose the participants. Since 1998, annual follow-ups have been performed in this population by utilizing interviews and selected exams. The fieldwork staff members were trained and certified before each examination.

The Bambuí Cohort Study was approved by the Ethics Committee of the Oswaldo Cruz Foundation in Rio de Janeiro, Brazil in 1996, and the present investigation was approved by the Ethics Committee of the Oswaldo Cruz Foundation in Belo Horizonte, Brazil in 2006. All of the participants provided full-informed written consent.

Study participants

Of the 1,742 residents aged 60 years or more, 1606 (92.2%) were interviewed and 1496 (85.9%) were physically examined (blood sample, laboratory tests, physical measurement and electrocardiogram) according to the cohort study baselines. All of the participants (N = 1,408) who had either a cognitive status measured by Mini-Mental State Exam (MMSE) or blood collected for *APOE* genotyping were selected for inclusion in the present study.

Measures

Cognitive status

The study questionnaire includes a standard Brazilian version of the MMSE.[14]. MMSE is a widely used instrument for assessing cognitive impairment.[15] However, its application to research

is much broader; it is specifically used to measure cognitive function in clinical and epidemiological studies of cognitively intact individuals. In the Brazilian validation study, some of the questions were modified according to their relevance to the target population.[16] In the orientation section by Folstein, questions regarding the season of the year, building and floor were replaced with period of the day, room, and address. In the registration and recall section, the words used were "cat", "tree", and "guitar". In the attention and calculation section, serial fives replaced serial sevens, and spelling "world" backward was replaced by "Maria," which is more commonly used in the Brazilian culture.

The MMSE score is influenced by education,[17] and there is no consensual validated cutoff point to be used for individuals with a low education. Because a low education level predominated in the study population, cognitive status was categorized by the percentile distribution of the MMSE. In this study, we used the cutoff point 21/22 (out of 30) that corresponded to the lower quartile of the MMSE score distribution.[18]

Apoliprotein E

Genomic DNA for *APOE* genotyping was extracted from blood samples using the Wizard® Genomic DNA Purification System (Promega, Madison, WI, USA). DNA samples were amplified by polymerase chain reaction (PCR), which was followed by digestion with HhaI and restriction fragment length polymorphism (RFLP) analysis as previously described.[19] The DNA samples were subjected to PCR with the following primers: forward 5' TAA GCT TGG CAC GGC TGT CCA AGG A 3' and reverse 5' ACA GAA TTC GCC CCG GCC TGG TAC AC 3'. The PCR conditions were denaturation at 95°C for 5 min.; 35 cycles of 95°C for 1 min., 60°C for 1 min., and 70°C for 2 min.; and a final extension at 72°C for 10 min. The RFLP analysis yielded the following patterns: E2E2, 91 and 83 bp; E3E3, 91, 48 and 35 bp; and E4E4, 72, 48 and 35 bp. Each of the heterozygote genotypes contained both sets of fragments from each *APOE* allele.

The following variables were additionally considered in this study: baseline age, gender, marital status, educational level, skin color, HDL cholesterol, triglycerides, systolic blood pressure (SBP) and depressive symptoms as assessed by General Health Questionnaire-12 (GHQ) using a cutoff of 4/5 to define case-level symptomatology as recommended for the Bambuí cohort study population.[20] The educational level was categorized as either 4 years or > 4 years of schooling. The interviewers classified the subjects' skin color based on photographs that were representative of individuals with different skin colors (white, light tan, dark tan, and black). After a 12-hour recommended overnight fast, the levels of HDL cholesterol and triglycerides were determined using commercial kits (Boehringer Mannhein Corp., Ingelheim, Germany) and an automated analyzer (Eclipse Vitalab, Merck, Netherlands) as described previously.[13] Three blood pressure (BP) measurements were taken on the right arm with an appropriately sized cuff using a mercury sphygmomanometer. The BP measurements were taken early in the morning following a 5 min. initial rest, and they were subsequently taken at 2 min. intervals and after 30 min. or more from the last instance of caffeine intake or cigarette smoked. BP was considered as the arithmetic mean of the second and third measurements. According to the Seventh Joint National Committee criteria,[21] the symptoms of common mental disorders were assessed by General Health Questionnaire-12 (GHQ) using a cutoff of 4/5 to define case-level symptomatology.[20]

Statistical Analysis

Unadjusted associations of *APOE* genotype with sociodemographic factors, biomarkers and mental health symptoms were evaluated using a Pearson x^2 test or ANOVA for assessing statistical significance of differences between the categorical and continuous variables, respectively. The unadjusted and adjusted odds ratios (ORs) were estimated by using logistic regression to assess the relationship between the *APOE* genotype and cognitive impairment. The analysis was based on five models. The crude association between the MMSE score and *APOE* genotype was estimated first, and it was then adjusted incrementally for gender, age and education (model 2); marital status and skin color (model 3); HDL, triglycerides and SBP (model 4); and GHQ score (model 5). In addition, gender and skin color interactions with *APOE* E4 carriers were tested in model 5. All of the analyses were performed with the STATA software package.

Results

Of the 1,606 cohort members, those who were included in the present analysis were the 1,408 baseline participants from whom cognitive status and *APOE* genotyping were determined (145 were excluded for refusing to perform blood tests, and 53 were excluded because they did not answer the MMSE). When comparing those individuals who were included in this analysis with those who were excluded, no difference was observed with respect to age (mean age = 69.0 ± 7.1 and 69.3 ± 7.4 years, respectively; p = 0.14) or gender (60.4% were men and 60.0% were women; p = 0.81).

The *APOE* allelic and genotypic distributions in this population were previously demonstrated.[22] The most frequent allele was E3 (80.0%), which was followed by E4 (13.5%) and E2 (6.5%); this distribution is representative of most western populations. The E3E3 genotype was the most common (63.4%), and it was followed by E3E4 (21.9%), E2E3 (11.5%), E4E4 (1.8%), E2E4 (1.4%), and E2E2 (0.1%). The allelic frequencies were within the Hardy-Weinberg equilibrium (p > 0.05).[22]

Table 1 shows the selected characteristics of the study population based on the presence of *APOE* E4. The overall mean MMSE score was 24.4 ± 4.7.The MMSE was lower among *APOE* E4 homozygotes when compared to the E2E4 and E3E4 heterozygotes. The other characteristics of the study participants did not differ significantly (p > 0.05) among the *APOE* genotypes.

Table 2 shows the unadjusted and adjusted OR for the associations between the *APOE* E4 carriers and cognitive impairment. *APOE* E4 homozygosity was significantly associated with a lower MMSE score, and this association remained after incrementally adjusting for age, gender, education, marital status, skin color, and for all other potential confounding variables (HDL, SBP, triglycerides and GHQ score) (OR = 2.98; 95% CI 1.15-7.71). Allele E4 homozygosity remained associated even when the participants who scored

Table 1 Baseline characteristics of participants by *APOE* genotype

CHARACTERISTICS Total	Total n = 1,408	*APOE* E4 NEGATIVE n = 1,054	*APOE* E4 HETEROZYGOTE n = 329	*APOE* E4 HOMOZYGOTE n = 25	p value
MMSE (mean/SD)	24.4 (4.7)	24.5 (4.7)	24.6 (4.6)	21.2 (4.9)	0.001
Gender (Female) (%)	60.4	61.0	59.6	48.0	0.394
Age (mean/SD)	69.0 (7.1)	69.0 (7.0)	69.2 (7.3)	67.8 (7.0)	0.641
Education ≥ 4 years (%)	35.1	36.0	32.8	24.0	0.287
Marital Status (%)					**0.766**
1. Married	50.2	49.7	51.6	50.0	
2. Single	9.8	10.3	8.6	4.2	
3. Divorced	5.2	5.6	3.8	4.2	
4. Widowed	34.8	34.3	36.0	41.7	
Skin Color (%)					**0.053**
1. White	60.3	59.7	62.9	48.0	
2. Light Tan	33.9	34.9	30.7	32.0	
3. Dark Tan	3.5	3.3	3.6	8.0	
4. Black	2.3	1.9	2.7	12.0	
Triglycerides (mean/SD)	151.1 (100.2)	150.7 (102.3)	152.7 (95.2)	145.1 (74.8)	0.910
HDL (mean/SD)	49.2 (15.1)	49.3 (14.9)	48.7 (15.7)	50.0 (16.1)	0.812
Systolic Blood Pressure (mean/SD)	137.3 (22.6)	137.1 (23.0)	137.3 (20.7)	141.8 (28.6)	0.594
GHQ ≥ 5 (%)	37.7	38.6	35.4	33.3	0.555

MMSE: Mini-Mental State Exam; GHQ: General Health Questionnaire-12

Table 2 Association of *APOE* E4 and cognitive impairment

Allele E4	Score MMSE ≥ 22	Score MMSE < 22	OR (CI 95%) Not adjusted	Adjusted for Age, Sex, Education	Adjusted for Age, Sex, Education, Marital Status, Skin Color	Adjusted for Age, Sex, Education, Marital Status, Skin Color, Triglycerides, HDL, Systolic Pressure	Adjusted for Age, Sex, Education, Marital Status, Skin Color, Triglycerides, HDL,Systolic Pressure, GHQ
0	824	207	1.0	1.0	1.0	1.0	1.0
1	255	64	0.99 (0.73 - 1.36)	0.92 (0.65 - 1.28)	0.94 (0.65 - 1.34)	0.95 (0.66 - 1.37)	0.95 (0.66 - 1.38)
2	14	11	3.12 (1.39 - 6.99)	2.99 (1.22 - 7.32	2.91 (1.13 - 7.50)	2.81 (1.09 - 7.28)	2.98 (1.15 - 7.71)

below the 5th percentile (cutoff 13/14) were removed from the analysis, minimizing the chance of masking the results with the undiagnosed AD subjects.

Although gender and skin color interactions with *APOE* E4 carriers were tested in the final model, the heterogeneity between the genders (p = 0.92) or among the skin colors (p = 0.31) was not statistically significant (data not shown).

Discussion

This study provides epidemiological evidence that *APOE* E4 homozygosity is associated with cognitive impairment in a population of Brazilian community-dwelling elderly, and the observed association persisted after carefully controlling for potential confounding variables.

To our knowledge, the current study is the first to evaluate the association of *APOE* E4 presence and cognitive status in a large, population-based cohort in Brazil. The strengths of this study include the following: (1) the use of an MMSE score percentile distribution in the absence of comparable cutoff points in the Brazilian population; (2) a large study population with a higher response rate (87.6%) of Brazilian elderly in a non-isolated population; (3) the adjustment for a range of potential confounders, including skin color, HDL and depression symptoms. The cross-cultural equivalence of the MMSE is concerning; however, to overcome this problem, we used a culturally adapted version of the MMSE that performed well in the studied population.[23]

Although studies investigating the association of *APOE* E4 carriers and cognitive status in community-living elderly are rare in Latin America, previous studies worldwide have suggested that an association exists between *APOE* E4 and cognitive performance. In a recent meta-analysis,[24] 38 studies showed that this association in heterozygous E4 carriers was not significantly different from non-carriers. However, the E4E4 homozygotes exhibited significantly poorer performance when compared with non-carriers; these results were confirmed in our study.

The findings from our study are most comparable with several studies that observed greater deficits in cognitive performance among *APOE* E4 homozygotes. Yaffe et al.[25] demonstrated in 1,750 cognitively intact, community-dwelling women aged 65 years and older in the United States that after adjusting for age, education, presence of a severe tremor, and depression, the MMSE reduction was 0% for individuals lacking APOE E4, 1.9% for *APOE* E4 heterozygotes, and 3.7% for *APOE* E4 homozygotes. In a cross-sectional exploratory study of cognitively normal residents (between 30 and 70 years of age), Caselli et al.[26] established that memory declined on all eight memory measures (including MMSE) in *APOE* E4 homozygotes. Memory declined on two of the eight measures in the heterozygotes, and it declined on one of eight in the non-carriers. Moreover, our study evaluated a larger sample than the only other Brazilian study[12], which failed to demonstrate an association between the *APOE* E4 allele and cognitive status.

The *APOE* E4 allele is known to represent a genetic risk factor in many populations, and it is considered as the most important susceptibility factor for AD development. Heterozygous *APOE* E4 carriers are at a 3- to 4-fold increased risk of becoming demented with AD, and *APOE* E4 homozygotes are at a 10- to 12-fold increased risk of developing AD.[27] In a recent study of a Scotland cohort longitudinal follow-up,[28] the findings indicated that the *APOE* E4 status is associated with cognitive changes in old age; they also suggested that the influence of allelic status may be restricted to specific domains of cognitive functioning. This study is consistent with our findings with respect to the observed significant relationship between the *APOE* E4 allelic status and MMSE performance.

In conclusion, in the analyzed population, cognitive impairment was associated with homozygosity of the E4 allele of the *APOE* gene but not with heterozygosity, indicating a likely gene dosage effect. Further studies are needed to evaluate the complex role of genetic markers, such as the *APOE* gene, on cognitive performance in Brazilian elderly individuals. Data from a prospective analysis of this population could help to elucidate our findings.

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Disclosure

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All of the authors participated in the study concept and design. SRQ-S and EC-C, participated in the revision of literature, analysis, and interpretation of the data and drafted the manuscript. MFL-C, EU and JOAF participated in the analysis and interpretation of the data and revision of the manuscript. EHM participated in the genotyping procedures and analyses. All of the authors critically reviewed the paper for important intellectual content.

** Modest*

*** Significant*

**** Significant. Amounts given to the author's institution or to a colleague for research in which the author has participation, not directly to the author.*

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