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Apolipoprotein E polymorphism distribution in an elderly Brazilian population: the Bambuí Health and Aging Study

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Abstract

Apolipoprotein E (*ApoE*) is one of the most extensively studied genes in the context of aging, but there are few population-based studies on *ApoE* polymorphism in the elderly in developing countries. The objective of the present study was to assess *ApoE* allele and genotype distribution in a large elderly community-based sample and its association with age, sex and skin color. Participants included 1408 subjects (80.8% of all residents aged ≥60 years) residing in Bambuí city, MG, Brazil. The DNA samples were subjected to the polymerase chain reaction amplification, followed by the restriction fragment length polymorphism technique, with digestion by *Hha*I. Analysis was carried out taking into consideration the six *ApoE* genotypes (ε3/ε3, ε3/ε4, ε2/ε3, ε4/ε4, ε2/ε4, and ε2/ε2), the three *ApoE* alleles, and the number of *ApoE*4 alleles for each individual. The ε3 allele predominated (80.0%), followed by ε4 (13.5%) and ε2 (6.5%). All six possible genotypes were observed, the ε3/ε3 genotype being the most frequent (63.4%). This distribution was similar to that described in other western populations. Sex was not associated with number of *ApoE*4 alleles. Black skin color was significantly and independently associated with the presence of two *ApoE*4 alleles (age-sex adjusted OR = 7.38; 95%CI = 1.93-28.25), showing that the African-Brazilian elderly have a high prevalence of the ε4 allele, as observed in blacks from Africa. No association between number of *ApoE*4 alleles and age was found, suggesting the absence of association of *ApoE* genotype with mortality in this population.

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Introduction

The search for genes and mutations potentially linked to diseases and aging itself has revealed many candidates. One of the genes most extensively studied in the context of aging has been the apolipoprotein E gene (*ApoE*), located on chromosome 19, which encodes for a protein that participates in the regulation of lipid metabolism (1-3).

The *ApoE* gene is polymorphic, containing single-nucleotide polymorphisms, which are mutations leading to changes in a single nucleotide base in the DNA sequence of the gene,

eventually causing changes in the amino acid sequence of the protein. Studies have shown that there are three common *ApoE* alleles in populations throughout the world, known as ε2, ε3, and ε4, giving rise to 6 genotypes (ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, and ε4/ε4). Allelic frequencies vary widely, with ε3 being the most common (wild type), while ε4 is more frequent in certain populations from Africa (4-6), northern Europe (5,7), Oceania (7), and in native Americans (8,9).

ApoE has multiple other roles in addition to its role in lipid metabolism (10) and the presence of the ε4 allele has been consistently linked to the development of Alzheimer's disease (11). This allele has also been associated with coronary heart disease (12, 13) and cerebrovascular disease (14,15) in some studies but not in others (16,17). In addition, *ApoE* has been studied in the context of mortality, but the results of these studies are controversial (18-24).

Community-based studies on *ApoE* polymorphism conducted in well-defined Brazilian populations are scarce (25), and all of them were small, involving populations of less than 500 subjects (8,9,25-30). The present study describes *ApoE* allele and genotype distribution in an elderly community-based sample of 1408 subjects, and its association with age, gender and skin color.

Material and Methods

Study area

The city of Bambuí (approximately 15,000 inhabitants) is situated in the Southwestern region of the State of Minas Gerais. Cerebrovascular diseases constitute the main cause of death in the population aged ≥60 years, followed by Chagas' disease and ischemic heart diseases. The Bambuí Health and Ageing Study (BHAS) is a population-based cohort study of older adults which is being conducted in Bambuí since 1997. In this report, we analyze data collected at the baseline of this study.

The Bambuí cohort study was approved by the Ethics Committee of the Oswaldo Cruz Foundation in Rio de Janeiro in 1996, and the present project was approved by the Ethics Committee of the Oswaldo Cruz Foundation in Belo Horizonte in 2006. All participants gave informed written consent.

Study population

From November to December 1996, a census was conducted in Bambuí to identify participants for the baseline study. Every person aged 60 years or older (N = 1742) was invited to take part in the study. Of these, 1606 (92%) were interviewed for risk factors, and 1496 (85.9%) had blood samples drawn for genomic DNA extraction. The latter subjects comprise the sample for the present study. Additional details have been reported elsewhere (31).

DNA extraction, PCR amplification and RFLP genotyping

Genomic DNA was extracted from the blood samples using the Wizard genomic DNA extraction kit (Promega, Madison, WI, USA). Samples were stored at -70ºC until further use. *ApoE* genotyping was carried out as described by Hixson and Vernier (32), with slight modifications. The DNA samples were subjected to the polymerase chain reaction amplification, using 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'. Polymerase chain reaction conditions were denaturation at 95ºC for 5 min, followed by 35 cycles of 95ºC for 1 min, 60ºC for 1 min, and 70ºC for 2 min, and a final extension at 72ºC for 10 min. The amplified DNA was subjected to the restriction fragment length polymorphism technique, with digestion by *Hha*I, generating the following patterns: ε2ε2, 83 and 91 bp; ε3ε3, 91, 48, and 35 bp, and ε4ε4, 72, 48, 35, and 19 bp.

These fragments were visualized on 4% agarose gels, instead of polyacrylamide gels as described in the original article.

Variables

In the present study, the dependent variable was the single-nucleotide polymorphism of the *ApoE* gene. Analysis was carried out taking into consideration the three *ApoE* alleles, the six *ApoE* genotypes (ε3/ε3, ε3/ε4, ε2/ε3, ε4/ε4, ε2/ε4, and ε2/ε2) and the number of *ApoE*4 alleles for each individual (0, 1 or 2 alleles).

Independent variables included age (60-69, 70-79, and ≥80 years), sex and skin color. Interviewers classified the subjects based on photographs representative of individuals with different skin colors (white, light brown, dark brown, and black).

Statistical analysis

Statistical analysis was based on Pearson's chi-square test and on multinomial regression (33). Allele frequencies were estimated by gene counting. Hardy-Weinberg equilibrium expectations were tested by using a chi-square goodness-of-fit test. The statistical analysis was performed using the Stata version 7.0 software (Stata Corporation, College Station, TX, USA).

Results

Of the 1606 BHAS cohort members, 1408 (557 males and 851 females) could be genotyped and participated in this study, their mean age being 69.3 years (SD = 7.2). The participants in this study were similar to non-participants regarding age (P = 0.999), sex (P = 0.365), and skin color (P = 0.063).

The *ApoE* allele and genotype distribution in the study population is shown in Figure 1. The most frequent allele was ε3 (80.0%), followed by ε4 (13.5%), and ε2 (6.5%). The distribution of *ApoE* alleles was within Hardy-Weinberg equilibrium (P > 0.05). All six possible genotypes were observed: the ε3/ε3 genotype predominated (63.4%), followed by ε3/ε4 (21.9%), ε2/ε3 (11.4%), ε4/ε4 (1.8%), ε2/ε4 (1.4%), and ε2/ε2 (0.1%).

There was no statistically significant difference (P = 0.311) in allele or genotype distribution among the various age groups (Table 1). The ε3 allele (79.4, 81.4, and 79.3%) and the ε3/ε3 genotype predominated in all age groups (61.8, 66.6, and

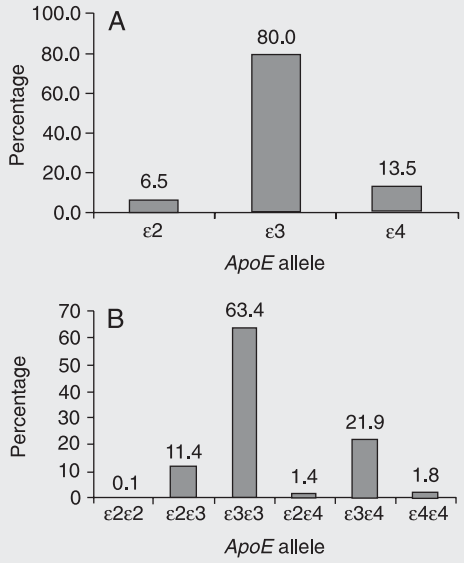

Figure 1. *ApoE* allele (A) and genotype (B) distribution among 1408 elderly participants at baseline of the Bambuí Health and Aging Study (Brazil).

Table 1. Apolipoprotein E (*ApoE*) allele and genotype distributions among 1408 elderly participants at baseline of the Bambuí Health and Aging Study by age group (Brazil).

ApoE	Age group		
	60-69 years	70-79 years	≥80 years
Allele			
ε2	110 (6.6%)	58 (6.7%)	15 (5.5%)
ε3	1328 (79.4%)	707 (81.4%)	219 (79.3%)
ε4	234 (14.0%)	103 (11.9%)	42 (15.2%)
Genotype			
ε2/ε2	0 (0.0%)	1 (0.2%)	0 (0.0%)
ε2/ε3	102 (12.2%)	47 (10.8%)	12 (8.7%)
ε3/ε3	517 (61.8%)	289 (66.6%)	86 (62.3%)
ε2/ε4	8 (1.0%)	9 (2.1%)	3 (2.2%)
ε3/ε4	192 (23.0%)	82 (18.9%)	35 (25.4%)
ε4/ε4	17 (2.0%)	6 (1.4%)	2 (1.4%)

Data are reported as number with percent in parentheses. *ApoE*: P = 0.481, genotype: P = 0.311 (Pearson's chi-square test).

62.3% among subjects aged 60-69, 70-79 and ≥80 years, respectively).

The distribution of the number of *ApoE*4 alleles according to demographic characteristic is shown in Table 2. A significant association between number of ε4 alleles and skin color was found (P = 0.023), but no associations with age (P = 0.437) or sex (P = 0.394) were observed. The prevalence of two ε4 alleles among black and dark brown subjects was 9.1 and 4.1%, respectively, while among light brown and white subjects the prevalence was less than 2%. The association between black skin color and two ε4 alleles was strong and independent of sex and age (OR = 7.38; 95%CI = 1.93-28.25).

Discussion

The results of the present study show that ε3/ε3 was the most frequent genotype and ε3 was the most frequent allele in the study population. These results are in agreement with previous observations that ε3 is the most common allele worldwide (5,7).

Regarding the ε4 and ε2 alleles, some differences in distribution have been reported. In Europe, studies have described a north to south cline in the ε4 allele, with a higher frequency in the northern region and a lower frequency in the southern region (7,34,35). The highest frequencies of ε4 have been described in Nigerians (4), Sub-Saharan Africans (5), South Africans (6), Inuit from Greenland (7), Finns (7), and native Americans (up to 47% in Brazilian natives) (8). ε2 is the least frequent allele, being completely absent from certain populations, in particular from several Native American tribes (8,9,26). In the present study, the prevalence of the ε4 and ε2 alleles was 13.5 and 6.5%, respectively.

In Brazil, studies carried out in children have found similar allele distributions. In Recife city (northeastern Brazil), allele distribution among 414 children ascertained at a pediatric hospital was as follows: ε3, 77%; ε4, 17%, and ε2, 6% (27). In Fortaleza city, also in northeastern Brazil, the corresponding findings among 72 shantytown children were 77.1, 14.6, and 8.3%, respectively (28). Previous studies of *ApoE* polymorphism in small samples of native Brazilian and South Ameri-

Table 2. Association of the number of *ApoE*4 alleles among 1408 elderly participants at baseline of the Bambuí Health and Aging Study with selected demographic characteristics (Brazil).

Variables	Number of *ApoE*4 alleles				
	None N (%)	One N (%)	Two N (%)	One OR (95%CI)*	Two OR (95%CI)*
Age group (years)					
60-69 years	619 (74.1%)	200 (23.9%)	17 (2.0%)	1.00	1.00
70-79 years	337 (77.6%)	91 (21.0%)	6 (1.4%)	0.83 (0.62-1.10)	0.69 (0.27-1.79)
≥80 years	98 (71.0%)	38 (27.5%)	2 (1.5%)	1.17 (0.78-1.76)	0.73 (0.16-3.24)
Gender					
Female	643 (75.6%)	196 (23.0%)	12 (1.4%)	1.00	1.00
Male	411 (73.8%)	133 (23.9%)	13 (2.3%)	1.05 (0.82-1.36)	1.65 (0.74-3.67)
Skin color					
White	630 (74.2%)	207 (24.4%)	12 (1.4%)	1.00	1.00
Light brown**	368 (77.1%)	101 (21.2%)	8 (1.7%)	0.84 (0.64-1.10)	1.11 (0.45-2.76)
Dark brown**	35 (71.4%)	12 (24.5%)	2 (4.1%)	1.04 (0.53-2.04)	2.87 (0.61-13.35)
Black	21 (63.6%)	9 (27.3%)	3 (9.1%)	1.30 (0.59-2.89)	7.38 (1.93-28.25)

ApoE = apolipoprotein E; OR = odds ratio; 95%CI = confidence interval at 95%. Age group: P = 0.437, sex: P = 0.394, skin color: P = 0.023 (Pearson chi-square test).
*Adjusted by multinomial logistic regression for the variables listed in the table (absence of ε4 allele was the reference group). **Light brown: "moreno"; dark brown: "mulatto".

can populations (8,9,26,29) found a highly heterogeneous distribution of alleles with a predominance of ε3 (frequency range: 51-98%), followed by ε4 (0-47%) and ε2 (0-4%).

Regarding older subjects, two Brazilian studies were carried out in the State of Rio Grande do Sul, in the south of Brazil (25,30). One involved a random sample of 64 subjects aged 80 years and older from a population of Italian descent residing in the city of Veranópolis. *ApoE* allelic frequencies were ε3, 84%; ε4, 11%, and ε2, 5%, and only four genotypes were observed: ε3/ε3 (70%), ε3/ε4 (22%), ε3/ε2 (6%), and ε2/ε2 (2%) (25). The other study involved 252 Caucasian volunteers ≥50 years of age residing in the city of Gravataí. *ApoE* allelic frequencies for this population were ε3, 76.7%; ε4, 16.2%, and ε2, 7.1%, with five genotypes (ε2 homozygotes were absent), ε3/ε3 being the most frequent (61.3%), followed by ε3/ε4 (24.3%) (30).

The allelic frequencies found in the present study are similar to the distributions found among older adults from south Brazil (25,30), even though the population from Bambuí is miscigenated and somewhat different from the populations in these studies which have a more strict European ascendance.

Reports that have included blacks from Africa (4-6) have suggested that there may be a higher frequency of the ε4 allele in blacks but, to the best of our knowledge, there are no population-based studies of *ApoE* polymorphisms in African-Brazilians. In our study, we found a gradient in the prevalence of ε4 homozygotes when genotypes were compared by skin color, with the highest prevalence among black-skinned individuals and the lowest among white-skinned subjects. Black-skinned individuals from this sample were significantly more likely to be ε4 homozygotes compared to white-skinned individuals. Among the dark-brown-skinned individuals the prevalence of ε4 homozygotes was twice as high as among white-skinned subjects, but this difference was not statistically significant. Those results could be the consequence of the greater ε4 prevalence mentioned above among African subjects, groups of which were brought to Brazil by the Portuguese during the 15th to 18th centuries as slaves, and which now form an important part of the Brazilian gene pool (8).

Several studies have investigated the association between ε4 allele and age. If an association of ε4 and mortality existed, one would expect to find a lower prevalence of this allele among the very old. Previously published results have been controversial, with some investigators reporting lower frequencies of ε4 among the very old (19-21), a finding which was not replicated by others (22-24). In the present study, we did not identify an association between ε4 allele prevalences and age.

This paper presents the largest population-based study on *ApoE* distribution carried out in Brazil, involving 1408 individuals who represent a well-defined target population. The results of the present study showed a distribution of *ApoE* alleles and genotypes similar to those observed in other western populations. The distribution of *ApoE* alleles was influenced by skin color, showing that the African-Brazilian elderly in the study population have a high prevalence of the ε4 allele, as observed in blacks from Africa (4-6). The distribution of *ApoE*4 alleles was not influenced by age, suggesting the absence of association with mortality in the study population.

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