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Association of *ApoE* polymorphisms with prevalent hypertension in 1406 older adults: the Bambuí Health Aging Study (BHAS)

A.K. Fuzikawa[1], S.V. Peixoto[1], M. Taufer[2], E.H. Moriguchi[3] and M.F. Lima-Costa[1]

[1]Núcleo de Estudos em Saúde Pública e Envelhecimento (NESPE), Fundação Oswaldo Cruz, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil
[2]CMIC Brasil Pesquisas Clínicas, Porto Alegre, RS, Brasil
[3]Universidade Federal do Rio Grande do Sul, Universidade do Vale dos Sinos, Hospital Moinhos de Vento de Porto Alegre, Porto Alegre, RS, Brasil

Correspondence to: M.F. Lima-Costa, Laboratório de Epidemiologia e Antropologia Médica, Instituto René Rachou, Fundação Oswaldo Cruz, Av. Augusto de Lima, 1715, 30190-002 Belo Horizonte, MG, Brasil
Fax: +55-31-3295-3115. E-mail: lima-costa@cpqrr.fiocruz.br

Apolipoprotein E (*ApoE*) polymorphism influences lipid metabolism, but its association with arterial hypertension is controversial. The objective of this study was to examine the association between *ApoE* polymorphism and prevalent hypertension in a large unselected population of older adults. Participants from the baseline of the Bambuí Health Aging Study whose *ApoE* genes had been genotyped were selected for this study (N = 1406, aged 60-95 years). These subjects represented 80.7% of the total elderly residents in Bambuí city, MG, Brazil. Hypertension was defined as a systolic blood pressure ≥140 mmHg and/or a diastolic blood pressure ≥90 mmHg, or the use of anti-hypertensive medication. The exposure variable was the *ApoE* genotype as follows: ε3 carriers, ε3ε3; ε2 carriers, ε2ε2 or ε2ε3, and ε4 carriers, ε3ε4 or ε4ε4. Potential confounding variables were age, gender, traditional cardiovascular risk factors, uric acid, and creatinine levels. The prevalence of hypertension was 61.3%. Compared with the ε3 homozygotes, neither the ε2 nor the ε4 carrier status was associated with hypertension (adjusted prevalence ratios = 0.94, 95%CI = 0.83-1.07 and 0.98, 0.89-1.07, respectively). On the other hand, the ε2 allele carriers had lower LDL cholesterol levels (P < 0.001) and the ε4 carriers had higher LDL cholesterol levels (P = 0.036). This study provides epidemiologic evidence that the *ApoE* genotype is not associated with prevalent hypertension in old age.

Key words: Apolipoprotein E; Hypertension; Low-density lipoprotein cholesterol; Triglycerides; Lipid metabolism

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Introduction

There has been an increasing interest in apolipoprotein E (*ApoE*) polymorphisms as predictors of hypertension. *ApoE* plays a fundamental role in lipid metabolism, participating in the clearance of chylomicron remmants and very low-density lipoproteins (VLDL) by serving as a ligand for low-density lipoprotein (LDL) receptors (1). It is also an important determinant of intestinal cholesterol absorption (2) and plasma lipid levels (3). The *ApoE* gene is located on chromosome 19 (4,5) with three common alleles, termed ε2, ε3 and ε4. The ε4 allele has consistently been linked to increases in LDL cholesterol levels (6,7). The ε2 allele has been reported to be linked with higher triglyceride levels in some (6,8), but not all studies (7).

Although *ApoE* genotype is involved in lipid metabolism, its association with arterial hypertension is controversial. Nevertheless, only two previous studies (9,10) involved populations of more than 1000 subjects, few studies have been carried out in representative community-

based samples, and few studies have considered traditional risk factors as potential confounders for the association between *ApoE* and hypertension or blood pressure level (9-21). Aditionally, few studies focused on the influence of *ApoE* genotype on hypertension in older individuals (9,12-14).

The primary objective of this study was to investigate the effect of the *ApoE* polymorphisms on the prevalence of hypertension in a population of 1406 community-dwelling older adults, considering several potential confounding factors in the analysis. A secondary objective was to examine the association between *ApoE* polymorphism and systolic blood pressure, diastolic blood pressure, high-density lipoprotein (HDL) cholesterol, LDL cholesterol and triglyceride levels.

Material and Methods

The Bambuí Health Ageing Study (BHAS)

The BHAS is a population-based cohort of older adults, which has been carried out in Bambuí city (~15,000 inhabitants), which is situated in Minas Gerais State, Southeastern Brazil. The cohort baseline was established in 1997. From November to December 1996, a complete census was carried out by the research team for identification of participants. Of the 1742 inhabitants aged 60 years or older by January 1, 1997, 1606 (92.0%) participated. The mean age of the cohort participants was 69.3 years (range: 60-95 years), and 60.0% were women. White skin color was predominant (60.4%), followed by light brown ("moreno", 33.3%), dark brown ("mulato", 3.6%), and black (2.7%) (22). The prevalence of hypertension (61.5%) was close to that described for the North American elderly (~60%) (23), as well as the prevalence of some traditional risk factors (24). A detailed description of the study design and methods has been published elsewhere (25).

Study participants

All of the 1406 cohort members who had their blood pressure (BP) measured at baseline and whose *ApoE* was genotyped were selected for the present study. The participants in this study were similar to non-participants in relation to age (P = 0.999) and gender (P = 0.365).

Measurements

Three measurements of systolic (SBP) and diastolic (DBP) were taken on the right arm with an appropriately sized cuff using a mercury sphygmomanometer. BP measurements were taken early in the morning, after a 5-min initial rest and subsequently at 2-min intervals between each measurement, and after 30 or more min of the last 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 (26), hypertension was defined as SBP ≥140 mmHg and/or DBP ≥90 mmHg or the use of anti-hypertensive medication. Current use of anti-hypertensive medication was ascertained by reviewing medication containers or prescription, and coding the medication as previously described (27).

Genomic DNA for *ApoE* genotyping was extracted from blood samples using the Wizard® Genomic DNA Purification System (Promega, Madison, WI, USA). DNA samples were then amplified by polymerase chain reaction (PCR), followed by digestion with *Hha*I, and restriction fragment length polymorphism analysis, as previously described (28). 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, followed by 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. Restriction fragment length polymorphism analysis yielded the following patterns: ε2ε2, 91 and 83 bp; ε3ε3, 91, 48 and 35 bp; ε4ε4, 72, 48 and 35 bp. Each of the heterozygote genotypes contained both sets of fragments from each *ApoE* allele.

Other variables considered included baseline age, gender, smoking, alcohol consumption, physical activity, family history of cardiovascular diseases, diabetes, traditional risk factors for cardiovascular diseases such as SBP, DBP, body mass index (BMI), and levels of LDL cholesterol, HDL cholesterol, triglycerides, creatinine (29), and uric acid (30). Current smokers were defined as those who had smoked at least 100 cigarettes during their lifetime and were still smoking. For alcohol consumption estimates, the subjects were shown cards with a representation of the amount of liquid corresponding to one drink for liquor, beer or wine. The alcohol consumption was calculated by multiplying the number of drinks by the frequency of imbibing in a week during the previous 12 months. Physical activity was defined as any exercise for 20-30 min at least three times a week, during leisure time in the previous 90 days. Diabetes was defined by a fasting blood glucose level ≥126 mg/dL and/or current use of insulin or oral antidiabetic drug treatment, as defined by the 2003 American Diabetes Association updated criteria (31). BMI (weight/height^2) was calculated from height and weight measurements. Levels of LDL cholesterol, HDL cholesterol, triglycerides, creatinine, and uric acid were determined after a 12-h recommended overnight fast, using commercial kits (Boehringer Mannhein, Germany) and an automated ana-

lyzer (Eclipse Vitalab, Merck, Netherlands), as described elsewhere (25).

All of the observers of the BHAS were trained and certified before each examination. The BHAS was approved by the Ethics Committee of the Oswaldo Cruz Foundation in Rio de Janeiro, RJ, Brazil, in 1996, and the present investigation was approved by the Ethics Committee of the Oswaldo Cruz Foundation in Belo Horizonte, MG, Brazil, in 2006. All participants gave full informed written consent.

Statistical analysis

The statistical analysis was performed by using the Stata version 9.1 software (Stata Corporation, College Station, TX, USA). Allele frequencies were estimated by gene counting. Hardy-Weinberg equilibrium expectations were tested by using a chi-square (χ^2) goodness-of-fit test. Non-adjusted analysis of the association between *ApoE* genotypes and alleles with prevalent hypertension was based on Person's chi-square test. Age-gender adjusted means and standard errors of the mean by *ApoE* genotype were calculated for baseline SBP, DBP, LDL cholesterol, HDL cholesterol, and triglyceride levels. Multiple linear regression was used to assess the association between *ApoE* genotype and the above mentioned parameters, after adjustments for age and gender. Poisson regression models (32) were used to estimate the prevalence ratio for hypertension. The models included age and gender in model 1, and age, gender, smoking, alcohol consumption, physical activity, family history, diabetes, LDL cholesterol, HDL cholesterol, uric acid, creatinine, and BMI in model 2. In the adjusted analysis, *ApoE* genotypes were classified into three groups: ε3 carriers (ε3ε3), ε2 carriers (ε2ε2, ε2ε3) and ε4 carriers (ε4ε4, ε3ε4). In each model, the homozygous ε3ε3 genotype was the reference group. Twenty subjects (1.4%) with the ε2ε4 genotype were excluded from the analysis because of the opposite effects of these two alleles on LDL cholesterol levels. Since no effect modification by gender was noted, all analyses were for both sexes. Statistical significance in the models was assessed using Wald χ^2 statistics.

Results

The prevalence of hypertension was 61.3% for the 1406 participants in the BHAS baseline. As found in most western populations, the ε3ε3 genotype was the most common (63.4%), followed by ε3ε4 (21.9%), ε2ε3 (11.5%), ε4ε4 (1.8%), ε2ε4 (1.4%), and ε2ε2 (0.1%). Allele frequencies were within the Hardy-Weinberg equilibrium expectations (P > 0.05). Additional baseline characteristics of the study participants are shown in Table 1.

Selected baseline characteristics of the BHAS population by *ApoE* genotypes, adjusted for age and gender are shown in Table 2. The mean LDL cholesterol was signifi-

Table 1. Selected baseline characteristics of the 1406 participants from the Bambuí Health and Aging Study (BHAS).

Characteristics	Percentage or mean (standard deviation)
Age (years)	69.1 (7.1)
Female gender	60.5%
Current smokers	18.1%
Alcohol consumption in the previous 12 months (>14 doses a week)	2.8%
Exercises lasting 20-30 min three times a week or more during the previous 3 months	12.5%
Family history of cardiovascular diseases	45.9%
Diabetes mellitus	14.1%
Systolic blood pressure (mmHg)	137.2 (22.6)
Diastolic blood pressure (mmHg)	83.6 (12.7)
Body mass index (kg/m^2)	25.0 (4.9)
LDL cholesterol (mg/dL)	155.0 (45.4)
HDL cholesterol (mg/dL)	49.1 (15.1)
Triglycerides (mg/dL)	151.1 (100.3)
Creatinine (mg/dL)	0.90 (0.30)
Uric acid (mg/dL)	5.3 (1.7)

Table 2. Age-sex adjusted analysis of the association between selected baseline characteristics of the Bambuí Health and Aging Study (BHAS) population and *ApoE* genotype.

ApoE genotype	N	Characteristics				
		Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)	LDL cholesterol (mg/dL)	HDL cholesterol (mg/dL)	Triglycerides (mg/dL)
ε3ε3	891	137.25 (0.75)	83.59 (0.42)	155.85 (1.50)	48.99 (0.49)	147.63 (3.34)
ε2ε2, ε2ε3	162	136.80 (1.76)	83.88 (0.99)	140.46 (3.56)*	50.95 (1.16)	167.11 (7.84)***
ε3ε4, ε4ε4	333	137.16 (1.23)	83.53 (0.69)	161.91 (2.47)**	48.20 (0.81)	153.63 (5.47)

Data are reported as means (standard error of the mean) adjusted for age and gender.
*P < 0.001; **P = 0.036; ***P = 0.022 compared to ε3ε3 (multiple linear regression).

cantly greater among the ε4 carriers (P = 0.036) and lower among the ε2 carriers (P < 0.001), in comparison to the homozygous ε3ε3 carriers. The mean triglyceride level was significantly greater among those with the ε2 allele (P = 0.022). No significant associations were found between *ApoE* genotype and SBP, DBP and HDL cholesterol levels.

The *ApoE* genotype and allele frequencies for the prevalence of hypertension are shown in Table 3. Both the *ApoE* genotype and the allele frequencies showed similar distributions among prevalent hypertension cases and non-cases.

The results of the adjusted analysis for the association between *ApoE* genotype and hypertension are shown in Table 4. Hypertension was not associated with the presence of either ε2 or ε4 alleles in the model adjusted by age and gender or in the model adjusted for several confounders.

Table 3. Non-adjusted analysis of the association between *ApoE* polymorphism and hypertension in the Bambuí Health and Aging Study (BHAS) population.

	Hypertension	
	Yes	No
ApoE genotype		
ε2ε2	1 (0.1%)	0 (0.0%)
ε2ε3	95 (11.0%)	66 (12.1%)
ε2ε4	13 (1.5%)	7 (1.3%)
ε3ε3	556 (64.5%)	335 (61.6%)
ε3ε4	179 (20.8%)	129 (23.7%)
ε4ε4	18 (2.1%)	7 (1.3%)
P = 0.538		
ApoE allele		
ε2	110 (6.4%)	73 (6.7%)
ε3	1386 (80.4%)	865 (79.5%)
ε4	228 (13.2%)	150 (13.8%)
P = 0.846		

Data are reported as number of subjects with percent in parentheses.
*P value: Person's chi-square test.

Table 4. Poisson regression models and prevalence ratio for hypertension in the Bambuí Health and Aging Study (BHAS) population, by *ApoE* genotype.

ApoE genotype	N	Hypertension prevalence (%)	Model 1 PR (95%CI)	Model 2 PR (95%CI)
ε3ε3	891	556 (62.4%)	1.0	1.0
ε2ε2, ε2ε3	162	96 (59.3%)	0.96 (0.83-1.09)	0.94 (0.83-1.07)
ε3ε4, ε4ε4	333	197 (59.2%)	0.95 (0.86-1.05)	0.98 (0.89-1.07)

Model 1: Adjusted for age and gender. Model 2: Adjusted for age, gender, smoking, alcohol consumption, physical activity, family history, diabetes, LDL cholesterol, HDL cholesterol, uric acid, creatinine, and body mass index. PR = prevalence ratio (95% robust confidence intervals).

Discussion

In this study, prevalent hypertension was not associated with *ApoE* polymorphisms in a large sample of Brazilian older adults. This lack of association was observed in the age-gender adjusted analysis and in the analysis adjusted for factors *a priori* believed to be potential confounders for the association between *ApoE* genotype and hypertension. Secondary analysis indicated that such associations were absent for both males and females (data not shown).

An association was observed between greater LDL cholesterol levels and the presence of the ε4 allele, as well as between lower LDL cholesterol levels and the presence of the ε2 allele. This observation is in agreement with other studies reporting a consistent relationship between the ε4 allele and greater levels of LDL cholesterol (6,7), as well as between the ε2 allele and lower LDL cholesterol levels (6). Higher triglyceride levels were found in ε2 carriers. This association was reported in some (6,8), but not all previous investigations (7). The association of the *ApoE* alleles with plasma lipid levels is a direct consequence of the role of ApoE protein in lipid metabolism. ApoE2 is metabolically impaired when compared to ApoE3 and ApoE4, due to its reduced interaction with cellular receptors, resulting in delayed clearance and accumulation of chylomicrons and VLDL remnants in the plasma. ApoE-mediated LDL conversion from VLDL remnants is also reduced. The net effect is an up-regulation of LDL receptors, higher plasma concentrations of triglyceride- and cholesterol-containing remnant particles, and lower plasma levels of cholesterol-rich LDL particles (1). *ApoE4* alleles have an opposite net effect compared to those of *ApoE2* (1,3).

There have been several studies of the association between the *ApoE* genotypes and prevalent hypertension, with inconsistent findings. Results from small prevalent case control studies have consistently described a positive relationship between the presence of the ε4 allele and hypertension or with greater BP levels (15-19). The results from cross-sectional studies have been more inconsistent. Four investigations carried out in an mixed population of younger and older adults reported i) lack of association between the *ApoE* genotype and hypertension in the USA (10) and in Tunisia (11), ii) a positive association between the ε2 allele and prevalent hypertension among male, but

not female, Japanese immigrants living in Los Angeles or Hawaii (20), and iii) a negative association between the presence of the ε4 allele and prevalent hypertension in young, but not older, Japanese (9). Three other cross-sectional studies included very old subjects. Two were carried out in Finland (12) and in Spain (14), and did not find significant association between *ApoE* genotype, prevalent hypertension (14), or mean SBP and mean DBP (12). The third study included Italian descendents living in the south of Brazil and reported a significantly lower DBP among the ε4 carriers, compared to the homozygous ε3ε3, but the mean SBP was similar in both groups (13). In addition, two cohort studies carried out with Japanese Americans (20) and North American males (21) reported contradictory results. In the present study, the mean baseline SBP and DBP, as well as prevalent hypertension, were not associated with the *ApoE* genotype.

This investigation benefits from a large number of subjects, the use of an unselected community-based population, and the use of double-blind data collection, as the technicians were unaware of the participants' *ApoE* or hypertension status. Selection and information bias are therefore unlikely, but prevalence bias is a potential limitation. The population investigated here consisted of older adults; therefore, the results may not be relevant to younger subjects.

In conclusion, this study confirms that the presence of the ε4 allele is associated with greater LDL cholesterol levels and the presence of the ε2 allele is associated with lower LDL levels and greater triglyceride levels. This investigation also provides epidemiologic evidence that *ApoE* genotype is not associated with prevalent hypertension in old age.

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