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Short Communication

Apoliprotein E genotype is associated with apoliprotein B plasma levels but not with coronary calcium score in very elderly individuals in primary care setting

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ABSTRACT

Background: Epidemiological surveys indicate the influence of polymorphisms of apolipoprotein (*apo*) *E* on plasma lipids and triglyceride-rich lipoprotein levels, with impact on atherosclerotic phenotypes.
Aim: We studied the association of classic genotypes of the *apoE* gene with clinical and biochemical risk factors for atherosclerosis in a segment of the very-old Brazilian individuals, with emphasis on the lipemic profile.
Methods: We performed cross-sectional analyses of clinical and laboratory assessments, including cardiac computed tomography, across *ε2*, *ε3 and ε4* carriers of the *apoE* gene with a convenience sample of 208 participants eligible for prevention against cardiovascular events.
Results: When non-*ε4* carriers were compared with *ε4* carrying subjects, lower levels of ApoB as well as ApoB/ApoA ratios were observed in the former group. Tests between *apoE* polymorphisms with other clinical/biochemical variables and those with arterial calcification showed no significant differences between groups.
Conclusion: The study suggests a possible atherogenic role of the *ε4* allele attributable to increased ApoB levels and ApoB/ApoA ratios among very-old subjects in primary care setting.

1. Introduction

Cardiovascular diseases (CVD) account for 48% of the deaths from noncommunicable diseases worldwide (Finegold et al., 2013). In Brazil, roughly one third of total deaths are represented by CVD (Sociedade Brasileira de Cardiologia et al., 2010), the main cause of deaths in the elderly population (Mansur and Favarato, 2012). Many clinical factors that contribute to CVD mortality such as obesity, hypertension, type-2 diabetes and dyslipidemia bear significant genetic contributors (Glass and Witztum, 2001). An increasing number of genetic polymorphisms have been associated with susceptibility to cardiovascular disease, mainly atherosclerosis, with emphasis on the important variability of the apolipoprotein (*apo*) *E* gene. Epidemiological surveys assessing the role of the *apoE* polymorphism on plasma lipids and triglyceride-rich lipoproteins (TRL) have shown that the presence of the *ε4* allele is associated with elevations in low density lipoprotein cholesterol (LDL-c), while the presence of *ε2* has the opposite effect on these particles (Alvim et al., 2010), possibly by virtue of the greater ability of the former and minor ability of the latter to act as ligands for specialized lipoprotein receptors (Demant et al., 1991) and hence an adaptative up-regulation of the receptor in *ε2* carriers (Alvim et al., 2010; Davignon et al., 1999). These gene–gene interactions are important because lipoproteins play a central role in the development of the atherosclerotic phenotype (Tiret et al., 1994), and ApoE is a key protein in the modulation of the catabolism of the most atherogenic particles (Alvim et al., 2010). There is evidence that ApoE has a profound impact on the ApoB metabolism (Demant et al., 1991) and on ApoB-related lipids and lipoproteins (Davignon et al., 1999). Recent genome wide association studies confirmed strong statistical association between *apoE* with CVD risk (Waterworth et al., 2010), primarily due to influences on levels of LDL-c (Smith et al., 2010) and total cholesterol

Abbreviations: ANCOVA, analysis of covariance; Apo, apolipoprotein; BMI, body mass index; CVD, cardiovascular disease; HDL-c, high density lipoprotein cholesterol; LDL-c, low density lipoprotein cholesterol; MANCOVA, multivariate analysis of covariance; SD, standard deviation; SPSS, Statistical Package For Social Sciences; TC, total cholesterol; TG, triglyceride; TRL, triglyceride-rich lipoprotein.

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(Aulchenko et al., 2009). Despite prospective studies indicating that the ApoB/ApoA-I ratio is a useful predictor of risk for myocardial infarction (MI) and other CVD (Sierra-Johnson et al., 2009), to our knowledge no studies sought to associate *apoE* genotypes with this aspect of the lipid metabolism of older adults. The very elderly are the age group that is growing the most rapidly in westernized societies such as those in Brazil (Nobrega et al., 2009) with an increasing number of individuals reaching this age stratum devoid of severe circulatory disorders (Freitas et al., 2012), and being eligible for primary prevention against major cardiovascular events. The goal of our work is to study the association of classic genotypes of the *apoE* gene with the atherosclerotic risk inherent to the lipemic profile of a segment of the very-old Brazilian population in primary care setting.

2. Methods

2.1. Subjects

Study subjects are participants in the ongoing Brazilian Study on Healthy Aging, which is a prospective cohort study that was started in December 2008 and designed to identify markers of cardiovascular risk in very elderly individuals eligible for primary prevention as published elsewhere (Freitas et al., 2011). For this proposal, non-institutionalized consecutive patients aged 80 years or over who have sought the outpatient clinic for preventive care and have never manifested myocardial infarction, stroke or peripheral arterial disease were enrolled. Additional selection criteria were the absence of autoimmune disease (including rheumatic disorders), chronic or recurrent infections, prior or current neoplastic disease, and use of steroidal or nonsteroidal anti-inflammatory drugs in the past 30 days.

After baseline measurements, all the participants were referred to the study outpatient clinic for prospective medical follow-up evaluations. Nonetheless, the results described herein derived from cross-sectional analyses with data at entry. The study was approved by the institutional research ethics committee and procedures were in accordance with the ethical standards of the Helsinki Declaration, with all participants having signed informed consent before enrollment.

2.2. Clinical inspection

All the subjects were submitted to assessments of total body mass (kg), body height (m), and blood pressure (mm Hg). Body mass index (BMI; kg/m^2) was defined as usual whereas waist circumference (WC; cm) was measured 2 cm above the umbilicus scar.

2.3. Biochemical analysis

After 12 h of overnight fasting, blood was collected and centrifuged at 5 °C, 4500 rpm for 15 min to separate plasma that was used exclusively to carry out the measurements of the metabolic variables analyzed herein: fasting glucose, glycated hemoglobin A (HbA) 1c, total cholesterol (TC), triglycerides (TG) and high density lipoprotein cholesterol (HDL-c). All glycemic and lipemic assessments were performed using reagents from Roche Diagnostics (Mannheim, USA) according to manufacturer recommendations and carried out by the same certified clinical laboratory. Low density lipoprotein cholesterol (LDL-c) was determined using the Friedewald equation (Friedewald et al., 1972). Assessments of C-reactive protein (highly sensitive, CardioPhase, Dade Behring, Marburg, USA), ApoA and ApoB (Behring Nephelometer BNII, Dade Behring, Marburg, Germany) were also performed.

2.4. Cardiac computed tomography

Computed tomography was performed in a 64-slice scanner (Aquillion 64, Toshiba, Ottawara, Japan). Axial slices of 3 mm thickness with 3 mm table-feed were acquired at 70% of R–R intervals with prospective electrocardiography triggering. Coronary artery calcification was defined as a minimum of 3 contiguous pixels with a peak Hounsfield unit density >130. Coronary artery calcifications were scored by a certified radiologist using the Agatston score to express its extent.

2.5. apoE genotyping

Whole blood was obtained during sampling for biochemical analysis and stored at −20 °C until use. Genomic DNA was purified according to standard extraction kits (QIAamp DNA Mini Kit, Qiagen, Brazil). To determine the classic genotypes for epsilon allelic variants of the *apoE* gene (*ε2*, *ε3* and *ε4*), amplifications based on a refractory mutation system (ARMS) for multiplex polymerase chain reactions were carried out using the method described by Donohoe et al. (1999), followed by electrophoresis of the amplification products in a 1.6% agarose gel. Each sample was run at least twice, and further checked only if the genotypes of the first two productive analyses were in conflict.

2.6. Statistical analysis

Violation of the Hardy–Weinberg equilibrium was tested using Fisher's exact test. The Kolmogorov–Smirnov test was used to verify normal distribution of data from continuous variables in a within-group approach. Whenever appropriate, data are expressed as means ± standard deviation (SD) or frequency (%). The Student's *t* test was used to compare the means of age and BMI across genotypes. Frequencies (e.g.: gender) were compared using the chi-square test. Analysis of covariance (ANCOVA) was used to analyze the variance in the main dependent variables across genotypes, with adjustment to age, BMI and gender. To test whether the intercorrelated nature of some dependent variables affected the parametric analyses outputs, MANCOVA was run to compare cholesterol-related metabolite values across groups, with the same adjustments. All the analyses were performed employing the Statistical Package for Social Sciences (SPSS) for Windows (version 17.0). A P value < 0.05 was considered significant.

3. Results

During the recruitment of patients, we assessed the clinical and biochemical characteristics of each subject at admission, along with the genotypes of the *apoE* gene. The allele frequencies of the *ε2*, *ε3* and *ε4* in the whole sample of 208 admitted individuals were 1.0%, 91.3% and 7.7%, respectively. Due to a low count of *ε2* carriers in the sample, these individuals were merged with the *ε3* homozygotes (non-*ε4* carries) for the purpose of comparison with *ε4* carriers. A single patient with the ε2/ε4 genotype was excluded from the analysis because of the putative opposite effects of these alleles in the phenotypes studied (Tiret et al., 1994), constituting a final sample of 207 subjects.

This sample showed a homogeneous pattern of baseline characteristics such as mean values of age and BMI and gender proportion across genotypes. Even so, these features were treated as covariates in the subsequent analyses. Regarding lipemic and clinical variables, ANCOVA revealed no differences in terms of plasma lipids (TC, LDL-c, HDL-c and TG) when non-*ε4* carriers were compared with *ε4* carrying subjects, albeit a non-significant trend towards lower LDL-c levels ($P = 0.08$) could be observed among the non-*ε4* carriers. Only in terms of serum ApoB levels and ApoB/ApoA ratios, were mean scores rendered significant ($P < 0.05$). There were no other clinical or laboratory differences observed between the groups (Table 1). MANCOVA for cholesterol-related metabolites as dependent variables (TC, LDL-c, HDL-c, TG, ApoB, ApoA and ApoB/ApoA) yielded similar results (Hotelling's Trace (7197) = 0.058), with greater serum ApoB levels ($F = 4.43$, $P = 0.036$) and ApoB/ApoA ratios ($F = 4.88$, $P = 0.028$) among the e4-carriers. The association of this structural polymorphism with the quantitative levels of ApoB, but not with ApoA, is suggestive of