APOA4 Polymorphism as a Risk Factor for Unfavorable Lipid Serum Profile and Depression: A Cross-Sectional Study

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Introduction: *APOA1/C3/A4/A5* gene cluster is closely involved in lipid metabolism, and its polymorphisms have been associated with coronary heart disease and lipid plasma levels. Here, we aimed to investigate associations of *APOC3* (3238C>G, −482C>T, 1100C>T) and *APOA4* (Gln360His, Thr347Ser) polymorphisms in 382 individuals from a cohort of a Longitudinal Brazilian Elderly Study with major age-related morbidities and with lipid and protein serum levels.

Materials and Methods: The whole sample was genotyped by polymerase chain reaction–restriction fragment length polymorphism. Descriptive statistics, logistic regression analysis, Student *t* test, deviation from Hardy-Weinberg, Bonferroni correction for multiple testing, and haplotype analyses were performed.

Results: Although *APOC3* 1100T allele carriers presented lower triglyceride and very low density lipoprotein levels than non–T carriers, these associations disappeared after Bonferroni correction ($P > 0.05$). Moreover, *APOA4* 360His allele was associated with depression ($P = 0.03$), increased triglyceride ($P = 0.035$) and very low density lipoprotein ($P = 0.035$) levels, and reduced HDL levels ($P = 0.0005$). Haplotype analyses found an association between His/C/C haplotype (Gln360His/−482C>T/1100C>T) with depression, but this result was due to Gln360His polymorphism.

Conclusions: Our data suggest that 360His allele might be a risk factor for depression and unfavorable lipid profile and depression for elderly people in the Brazilian population.

Key Words: *APOC-III*, *APOA-IV*, elderly population, HDL, triglycerides

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Aging is generally associated with increased predisposition to illness and death due to the altered homeostasis and the reduced responsiveness to environmental factors.[1] Diseases involving the cardiovascular system are the most important cause of mortality in most developed and in developing countries and cause around 32% of deaths in Brazil, particularly after the age of 45 years.[2] Increased plasma triglyceride (TG) levels and reduction of high-density lipoprotein/low-density lipoprotein (HDL/LDL) ratio are independent risk factors for atherosclerosis and cardiovascular disease (CVD).[3] *APOA1/C3/A4/A5* gene cluster, located on 11q23, is closely involved in lipid metabolism and has been associated with coronary heart disease,[4] obesity,[5] and with lipid plasma levels.[6]

APOC3 is a surface component of TG-rich lipoproteins and is a noncompetitive inhibitor of lipoprotein lipase.[7] APOC3 has been associated with CVD[8] and with higher TG levels.[9] Moreover, most of the therapies used in lowering TG, such as niacin, fish oil, and fibrates, are associated with a decrease in *APOC3* expression.[10] Common polymorphisms were identified in *APOC3* promoter (−482C>T – rs2854117), exon 3 (1100C>T – rs4520), and in 3′-unstranslated region (3238C>G – rs5128). These polymorphisms have been associated with hypertriglyceridemia[11] and susceptibility to CVD in European,[12] Chinese,[13] and Asian Indian populations.[11] *APOC3* 1100C>T polymorphism has not yet been studied in the Brazilian population.

APOA4, another apolipoprotein within this gene cluster, acts as a satiety signal[14] and has been proposed to be a protective factor for atherosclerosis.[15] Two polymorphisms in *APOA4* (Gln360His – rs5110 and Thr347Ser – rs675) have been characterized. It was shown that Gln360His polymorphism was associated with obesity and depression and that 360His allele carriers presented 2-fold increased risk for both morbidities.[16]

To establish whether polymorphisms of *APOC3* and *APOA4* might act as risk factors for some age-related diseases in a Brazilian elderly cohort, we assessed the association of *APOC3* 3238C>G, −482C>T, and 1100C>T polymorphisms and *APOA4* Gln360His and Thr347Ser polymorphisms and their haplotypes with morbidities (CVD, type 2 diabetes, hypertension, obesity, depression, dementia, and neoplasia), TG, total cholesterol, HDL, very low density lipoprotein (VLDL), LDL, creatinine, urea, albumin, glycated hemoglobin (Hb_{Ac1}), and fasting glucose serum levels.

MATERIALS AND METHODS

Population

The Elderly Longitudinal Study (EPIDOSO)[17] began in 1991 and originally involved 1667 people older than 66 years living in a community of São Paulo, Brazil. Subjects were clinically evaluated every other year and a subsample of 382 (261 females and 121 males) in wave 4 (2000–2001) was invited to participate in our study. The mean age of this population was 79.82 (5.31) years (age range, 66–97 years). This population was composed of individuals of European (89.2%), Japanese (3.3%), Middle Eastern (1.8%), and mixed and/or other (5.7%) origins.

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Clinical inquiries were performed to obtain information about previous diseases, current medication use, lifestyle, anthropometric, and blood pressure measurements. Physicians performed the physical examination, and blood samples were collected for laboratory procedures. The Research Ethics Committee of UNIFESP approved this study, and all participants gave us informed consent.

Individuals were considered positive for CVD when they self-reported previous CVD and were taking specific medication prescribed by physicians. Those using antihypertensive drugs or those with systolic blood pressure greater than 140 mm Hg or diastolic blood pressure greater than 95 mm Hg were considered positive for hypertension.[18] Those taking insulin or oral medication and those with fasting glucose equal to or greater than 126 mg/dL were considered positive for type 2 diabetes.[19] Neoplasia was considered positive when individuals self-reported previous diagnosis with confirmation in their medical record, with histologic results. Subjects with body mass index greater than 27 kg/m^2 were considered positive for obesity.[20] Cognitive function was evaluated by the Mini-Mental State Examination screening instrument validated for the Brazilian population.[21] A Mini-Mental State Examination score lower than 24 (/30) has 80% to 90% sensitivity and 80% specificity for discriminating low cognition level for healthy subjects.[21] Depression was characterized by a score higher than 5 in a validated Brazilian version of Older Americans Resources and Services.[22] Although some studies have shown that self-reported past history and medical records are usually concordant for selected medical conditions in the elderly,[23] past histories were only accepted when there was also evidence in physical examinations, eletrocardiogram, computed tomographic scan, or physician reports. Table 1 summarizes all descriptive characteristics of the sample.

Laboratory Examinations

Laboratory examinations were performed as routine examinations using commercial kits. Lipid and lipid fraction measurements were performed using enzymatic tests. Urea, creatinine, and albumin serum levels and fasting glucose were investigated by colorimetric, kinetic, and ultraviolet tests. Hb_{Ac1} was measured by high-performance liquid chromatography.[24] The coefficients of variation for these measurements ranged from 2% to 3.5%.

TABLE 1. Clinical Characteristics of the Study Population

Variables	n	No. Affected Individuals (%)	Mean (SD)
Sex, male/female	382	121/261 (31.7/68.3)	
Age, yr	382		79.82 (5.31)
CVD	382	83 (21.7)	
Type 2 diabetes	381	242 (63.5)	
Hypertension	380	318 (83.7)	
Obesity	308	127 (41.2)	
Depression	354	71 (20.1)	
Dementia	375	30 (8.0)	
Neoplasia	377	41 (10.9)	
Total cholesterol, mg/dL	247		215.90 (42.64)
Triglyceride, mg/dL	247		149.51 (71.32)
VLDL, mg/dL	241		28.58 (11.27)
HDL, mg/dL	246		54.36 (14.20)
LDL, mg/dL	239		131.85 (35.94)
Urea, mg/dL	239		40.44 (12.60)
Creatinine, mg/dL	238		0.95 (0.24)
Albumin, g/dL	238		4.04 (0.32)
Hb_{Ac1},%	339		5.77 (1.48)
Fasting glucose, mg/dL	348		99.05 (32.23)

Genotyping

Whole blood was collected into tubes containing 0.1% EDTA and genomic DNA extraction was performed using QIAamp DNA Blood Midi Kit (Qiagen, Germantown, MD), according to the manufacturer's protocol.

Primers for all amplification reactions were previously described.[16,25–28] Polymerase chain reaction mixtures were composed by 100 ng of genomic DNA, 0.8 μM of each primer (Integrated DNA Technologies, Coralville, IA), 0.1 mM dNTPs (Invitrogen, Carlsbad, CA), 2 mM $MgCl_2$, and 1 U of Taq DNA polymerase (LGC, São Paulo, Brazil) in 10% polymerase chain reaction buffer. Cycling conditions were as follows: denaturation at 94°C for 5 minutes, then 35 cycles of 94°C for 1 minute, 60°C for 30 seconds, and 72°C for 45 seconds, and a final extension at 72°C for 7 minutes. After amplification of the fragments for 3238C>G, −482C>T, 1100C>T, and Thr347Ser polymorphisms, amplicons were digested with 5 U of *Sac*I, *Msp*I, *Bst*EII, and *Hin*fI enzyme (New England Biolabs, Ipswich, UK), respectively, at 37°C for 4 hours.

Statistics

χ^2 tests, logistic regression analyses, and Student *t* test were performed using SPSS 16.0 (SPSS Inc, Chicago, IL). Genotype and allele frequencies were calculated by allele counting as described by Emery.[29] Genotype distributions were investigated according to the Hardy-Weinberg equilibrium. Logistic regression analyses were performed considering the morbidity as a dependent variable and allele, age and sex as covariables in the model. Odds ratio and 95% confidence interval were also calculated. Mean serum levels were compared between the 2 allele groups by Student *t* test ($\alpha = 0.05$). Bonferroni correction for multiple testing was performed. Haplotypes were estimated using HaploView[30] and Linkage Disequilibrium Analyzer (LDA) 31 software, and their associations with morbidities were tested by χ^2 test.

RESULTS

Minor allele frequencies for *APOC3* −482C>T, 1100C>T, and 3238C>G polymorphisms and *APOA4* Gln360His and Thr347Ser polymorphisms were 0.348, 0.265, 0.124, 0.056, and 0.237, respectively. −482C>T, 1100C>T, and Gln360His polymorphisms were within the Hardy-Weinberg equilibrium in this cohort ($P > 0.05$).

Our study did not show association of *APOC3* 3238C>G, *APOC3* −482C>T, *APOC3* 1100C>T, and *APOA4* Thr347Ser polymorphisms with any studied morbidity. On the other hand, *APOA4* 360His allele was associated with obesity and depression ($P = 0.038$ and $P = 0.006$, respectively). After Bonferroni correction for multiple comparisons, the association of 360His allele with depression remained ($P = 0.03$); however, the association of this allele and with obesity disappeared ($P > 0.05$). We observed that all subjects with dementia presented only 360Gln allele; hence, statistical analyses were not performed in this small subsample.

Our results showed that 1100T allele was associated with decreased TG ($P = 0.035$) and VLDL levels ($P = 0.041$), which lost significance after Bonferroni correction ($P > 0.05$). In addition, significant associations between 360His allele and

TABLE 2. Haplotype Frequencies Analyses of Nonaffected and Affected Individuals for Depression

Haplotypes		Depression		
Gln360His /−482C>T /1100C>T	Whole Sample, %	Nonaffected, %	Affected, %	*P*
Gln/C/C	0.465	0.453	0.491	0.4707
Gln/T/C	0.221	0.231	0.207	0.5892
Gln/C/T	0.139	0.148	0.107	0.2557
Gln/T/T	0.124	0.128	0.099	0.4046
His/C/C	0.048	0.039	0.095	**0.0138***

*$P < 0.05$.

reduced HDL and increased VLDL and TG levels were observed even after Bonferroni correction for multiple comparisons ($P = 0.0005$, $P = 0.035$, and $P = 0.035$, respectively).

We also performed haplotype analyses using HaploView[30] and LDA[31] software, and only polymorphisms within the Hardy-Weinberg equilibrium were considered. Although *APOA4* Gln360His and *APOC3* −482C>T were in strong linkage disequilibrium ($D' = 1.0$; $P = 0.0006$), *APOA4* Gln360His and *APOC3* 1100C>T ($D' = 0.75$; $P = 0.0262$) and *APOC3* −482C>T and *APOC3* 1100C>T ($D' = 0.19$; $P = 0.0135$) were in weak linkage disequilibrium. Haplotype analyses were composed of *APOA4* Gln360His, *APOC3* −482C>T, and *APOC3* 1100C>T polymorphisms, in this order. Among them, the least common in this population was His/C/C (4.8%), which was associated with depression ($P = 0.0138$; Table 2). Haplotypes involving *APOC3* polymorphisms (1100C>T and −482C>T) did not show any association with morbidity.

DISCUSSION

Studies conducted in an elderly population may clarify specific issues concerning the aging process. One of the major causes of death in adult and elderly populations is CVD, which is closely linked to lipid and apolipoprotein levels.[6]

Our study showed that allele frequencies of *APOC3* 3238C>G and *APOC3* −482C>T polymorphisms were comparable to those in another Brazilian adult sample.[32] Similar frequencies for *APOC3* 3238C>G,[33] *APOA4* Gln360His, and *APOA4* Thr347Ser[34] polymorphisms and lower frequencies for *APOC3*−482C>T and *APOC3* 1100C>T polymorphisms[35] were observed in populations of European ancestry.

All *APOC3* polymorphisms showed no association with any studied morbidity, confirming studies in European populations.[24,36] We found that *APOC3* 1100T allele was associated with reduced TG and VLDL levels, although these associations did not remain after Bonferroni correction. To our knowledge, this was the first study to investigate the *APOC3* 1100C>T polymorphism in a Brazilian elderly population. Therefore, further studies of this polymorphism in different populations are needed. The lack of association of *APOC3* 3238C>G and *APOC3* −482C>T polymorphisms with serum level variables in our sample has also been observed in European populations[32] and in Brazilian female children.[37]

We did not find any association of *APOA4* Thr347Ser polymorphism with morbidities or serum level variables, confirming other studies in European populations[28,36,38] and in Asian Indians.[22] Conversely, this polymorphism was associated with obesity-related traits in other adult Brazilian[39] and European populations.[40]

Although we have not found association between obesity and Gln360His polymorphism after Bonferroni correction, this result was found in another Brazilian population.[37] Despite this fact, it is worthy of note that His-allele frequency was higher in the affected group. In addition, our sample is an admixed population from Brazil and His-allele has been proposed as a marker of European admixture[41]; hence, it could be modifying our results. Concerning serum levels, we observed that 360His allele showed reduced HDL and higher VLDL and TG levels, which might be due to delayed postprandial clearance of TG-rich lipoprotein[42] and may be a risk factor for metabolic disease.

Moreover, Gln360His, a functional *APOA4* polymorphism, may alter the apolipoprotein structure and play a role in the pathophysiology of depression. However, this association should be tested in other populations.

We found that His/C/C haplotype was associated with depression and that none of the haplotypes presenting *APOA4* Gln allele were associated with any morbidity. When we analyzed only the *APOC3* −482C>T/1100C>T haplotypes, the association did not remain, which suggests that *APOA4* Gln360His polymorphism might be a risk factor for this disease in our population. Thus, the exclusion of *APOA4* Gln360His polymorphism from the haplotype analyses showed that *APOC3* polymorphisms were not involved in the development of depression, and the association between His/C/C haplotype and this disease might be due to the *APOA4* Gln360His polymorphism.

We previously reported in this same cohort an association of *APOA1* +83C>T polymorphism with obesity, and T allele was more frequently observed in subjects with hypertension in the presence of CVD.[5] Polymorphisms within *APOA1/C3/A4/A5* gene cluster are in linkage disequilibrium; therefore, other haplotype analyses may support our results. Some limitations of this study may include small sample size and the lack of ethnic markers. Although it would have been better to do the genotyping in the whole cohort (1667 individuals), which is a considerable sample size, only 382 participants of EPIDOSO remained in the study and were available for genetic investigation. Moreover, we could not genotype ethnic markers, which would have add interesting information to the study, because the Brazilian population is admixed and the proportion of ethnic groups varies among distinct Brazilian regions. Hence, we could find divergences, as reported by Mazzotti et al.[43] Therefore, we should have taken into account this covariable, although our sample was composed mainly of European descendants.

In conclusion, our study revealed that *APOA4* 360His allele has been associated with an unfavorable lipid profile and might be associated with depression in a sample of Brazilian elderly population.

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