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Short Communication

Association of *APOA1* and *APOA5* polymorphisms and haplotypes with lipid parameters in a Brazilian elderly cohort

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ABSTRACT. Apolipoproteins have an important role in lipid metabolism and transport. Polymorphisms in the *APOA1/C3/A4/A5* gene cluster have been associated with lipid alterations and cardiovascular diseases. We investigated *APOA1 Xmn*I, *APOA5* S19W, and *APOA5* -1131T>C polymorphisms in 377 individuals from a cohort of a longitudinal Brazilian elderly study. Allele frequencies, genotype distribution, and association with major morbidities as well as with lipids, creatinine, albumin, urea, glycated hemoglobin, and fasting glucose serum levels were investigated. Linkage disequilibrium and haplotype associations were also analyzed. This is the first time that haplotypes involving these polymorphisms were evaluated. Genotyping was performed by PCR-RFLP. Minor allele frequencies were 0.119, 0.071, and 0.158 for *Xmn*I, S19W, and -1131T>C polymorphisms, respectively. We found a significant association of the -1131C allele with low LDL-C levels. We also observed that *Xmn*I

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and S19W polymorphisms were in linkage disequilibrium. The C/G haplotype, which is composed of the wild-type allele of *Xmn*I and the minor allele of S19W, was associated with high total cholesterol serum levels in this elderly population. We conclude that the -1131T>C polymorphism and the C/G haplotype, including *Xmn*I and S19W polymorphisms, are associated with alterations in lipid levels and may be risk factors for cardiovascular disease in the Brazilian elderly.

Key words: *APOA1*/*A5* polymorphisms; Apolipoproteins; Haplotypes; Brazilian elderly population; LDL-C level; Total cholesterol level

INTRODUCTION

Alterations in lipid metabolism are highly associated with cardiovascular diseases (CVD). Elevated levels of total cholesterol, low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG) and low high-density lipoprotein cholesterol (HDL-C) levels are determinant risk factors for CVD (Hamon et al., 2006; Andrade et al., 2010). In 2009, three genome-wide association studies described new loci associated with serum lipid levels, including the *APOA1/C3/A4/A5* gene cluster, located on 11q23 (Aulchenko et al., 2009; Kathiresan et al., 2009; Sabatti et al., 2009).

APOA1 is the main apolipoprotein component of HDL-C, and an inverse relationship between HDL-C and APOA1 concentrations and risk for premature atherosclerosis has been reported (Srivastava and Srivastava, 2000). APOA5, another apolipoprotein, has been reported to be an important regulator of TG levels (Hubacek et al., 2004). Polymorphisms in *APOA1* and *APOA5* genes have been associated with lipid alterations and CVD (Hubacek et al., 2004; de Andrade et al., 2011).

We aimed to determine the allelic frequencies of three well-described single nucleotide polymorphisms (SNPs), namely *APOA1 Xmn*I (rs11216158), *APOA5* S19W (rs3135506), and *APOA5* -1131T>C (rs662799), as well as to investigate the association of these SNPs and their haplotypes with the morbidities (CVD, hypertension, type II diabetes, obesity, depression, and dementia) affecting an elderly Brazilian cohort and with serum levels of TG, total cholesterol, HDL-C, very low-density lipoprotein cholesterol, LDL-C, creatinine, urea, albumin, glycated hemoglobin, and fasting glucose.

MATERIAL AND METHODS

The population studied was a subsample of 377 subjects (257 females and 120 males) who participated in wave 4 (2000-2001) of the Elderly Longitudinal Study (EPIDOSO) (Ramos et al., 1998). Table 1 summarizes the characteristics of our sample. This population had a mean age of 79.76 ± 5.29 years (range 66-97 years) and was composed of individuals of European (89.2%), Japanese (3.3%), Middle Eastern (1.8%), and mixed or other (5.7%) origins. The Institutional Research Ethics Committee approved this study, and all participants or their representatives gave informed consent according to the Declaration of Helsinki. All clinical characterization of our sample, laboratory tests, and DNA extraction have been previously described (Chen et al., 2010).

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Table 1. Age, gender, serum levels, and morbidities affecting the sample studied of an Brazilian elderly cohort.

Variables	N	Number of affected individuals (%)	Means ± SD
Age (years)	377		79.76 ± 5.29
Gender	377	257 ♀ (68.2%)/120 ♂ (31.8%)	
Cardiovascular disease	377	83 (22.0%)	
Type II diabetes	376	240 (63.8%)	
Hypertension	375	313 (83.5%)	
Obesity	303	124 (40.9%)	
Depression	349	69 (19.8%)	
Dementia	370	39 (8.1%)	
Total cholesterol (mg/dL)	243		216.12 ± 42.23
Triglyceride (mg/dL)	243		150.06 ± 71.58
VLDL cholesterol (mg/dL)	237		28.67 ± 11.29
HDL cholesterol (mg/dL)	242		54.28 ± 13.89
LDL cholesterol (mg/dL)	235		132.05 ± 35.84
Urea (mg/dL)	235		40.36 ± 12.61
Creatinine (mg/dL)	234		0.95 ± 0.24
Albumin (g/dL)	234		4.04 ± 0.33
Glycated hemoglobin (%)	335		5.78 ± 1.48
Fasting glucose (mg/dL)	344		99.18 ± 32.37

N = number of consulted individuals; SD = standard deviation; VLDL = very-low density lipoprotein.

For *APOA1 Xmn*I polymorphism, a 331-bp fragment was amplified using the primers: forward: 5'-AGACGAGGGAAAGAAATGGGTGGA-3' and reverse: 5'-TGAACAGCATCTTACCAAGCAGGCA-3'. Primers used for the *APOA5* S19W polymorphism amplification were previously described (Pennacchio et al., 2002). PCR cycling conditions were 5 min at 94°C, followed by 35 cycles of 94°C for 30 s, 60°C (*Xmn*I) or 69°C (S19W) for 30 s, and 72°C for 45 s, and a final extension at 72°C for 7 min. PCR products were digested with 5 U *Xmn*I or 7.5 U *Eag*I (New England Biolabs) at 37°C for 16 h for *Xmn*I and S19W polymorphisms, respectively. *APOA5* -1131T>C genotyping was performed as described by Chen et al. (2006).

Descriptive statistics, logistic regression analysis, the χ^2 test, and the Student *t*-test were used. Linkage disequilibrium and haplotype association analyses were also performed. Details of the statistical analyses were described in our previous report (Chen et al., 2010).

RESULTS AND DISCUSSION

Minor allele frequencies were 0.119, 0.071, and 0.158 for *Xmn*I, S19W, and -1131T>C polymorphisms, respectively. The -1131T>C polymorphism deviated from Hardy-Weinberg equilibrium ($P < 0.05$) and was not included in the haplotype analysis.

We did not find any associations of *Xmn*I and S19W polymorphisms either with the serum lipid and protein analyzed or with the morbidities studied, in agreement with studies in other populations (Marcil et al., 1996; Martinelli et al., 2007). However, associations of the T allele of the *Xmn*I polymorphism with increased TG, total cholesterol, and LDL-C levels have been described (Dallinga-Thie et al., 1996; Hong et al., 1997). Regarding the S19W polymorphism, the G allele has been associated with higher plasma TG in several populations (Hubacek et al., 2004; Martinelli et al., 2007), as well as in another population from the South Brazil region (de Andrade et al., 2011). These controversial findings may be due to the heterogeneity of the study populations and to their different ethnic backgrounds.

Our data showed that *Xmn*I and S19W polymorphisms were in linkage disequilibrium ($r^2 = 0.042$; D' = 0.2908; P = 0.001). Upon analyzing the haplotypes, we found that the C/G haplotype, which is composed of the wild-type allele of *Xmn*I and the minor allele of S19W, respectively, was associated with higher serum total cholesterol levels (P = 0.048) compared to the C/C haplotype, which includes the wild-type alleles for both polymorphisms. Although this borderline association had a small effect, it must be considered since these findings suggest a synergistic effect of these SNPs on serum cholesterol levels. Hamon et al. (2006) showed that multiple SNPs within the *APOA1/C3/A4/A5* gene cluster did not have significant genotype effects when separately considered, but when combined to define two-SNP genotypes, they had significant effects on total cholesterol levels. Haplotype analyses can therefore provide additional information compared to the single SNP analyses, which may result in an underestimated contribution of genetic variation to changes in quantitative CVD risk factor traits.

Concerning the -1131T>C polymorphism, a previous study from our group did not show an association between C allele and morbidities (Chen et al., 2006). On the other hand, the present study found that C allele carriers [mean = 124.49; standard deviation (SD) = 39.86] showed lower LDL-C levels than did T allele carriers (mean = 133.56; SD = 36.20; P = 0.036). It has been shown that elevated LDL-C levels are strongly related to CVD (Andrade et al., 2010), suggesting that this polymorphism might act as a protective factor against CVD.

In conclusion, our study showed a significant association of the -1131C allele with lower LDL-C levels and of the C/G haplotype, concerning the *Xmn*I and S19W polymorphisms, with higher serum total cholesterol levels in a Brazilian elderly cohort. Caution must be taken at this time in interpreting these results, which need to be confirmed with studies in larger populations. Nonetheless, these data may lead to a better understanding on how genetic factors contribute to changes in serum lipid levels and may modify CVD risk in the Brazilian elderly population.

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