

Relevance of apolipoprotein E4 for the lipid profile of Brazilian patients with coronary artery disease

D.R.S. Souza¹, L. Nakachima¹,
R.B. Biagioni¹, M.A. Nakazone¹,
M.A.S. Pinhel¹, D.M. Trindade¹,
V.T. Mafra¹, L.H.B. Tácito²,
J.F.V. Martin², S. Pinheiro Júnior¹
and A.C. Brandão¹

¹Departamento de Biologia Molecular, ²Departamento de Clínica Médica,
Faculdade de Medicina de São José do Rio Preto, São José do Rio Preto, SP, Brasil

Abstract

Apolipoprotein E (apoE - $\epsilon 2$, $\epsilon 3$, $\epsilon 4$ alleles) plays a role in the regulation of lipid metabolism, with the $\epsilon 4$ considered to be a risk factor for coronary artery disease (CAD). We aimed to evaluate the apoE polymorphisms in Brazilians with CAD and their influence on the lipid profile and other risk factors (hypertension, diabetes mellitus, smoking). Two hundred individuals were examined: 100 patients with atherosclerosis confirmed by coronary angiography and 100 controls. Blood samples were drawn to determine apoE polymorphisms and lipid profile. As expected, the $\epsilon 3$ allele was prevalent in the CAD (0.87) and non-CAD groups (0.81; $P = 0.099$), followed by the $\epsilon 4$ allele (0.09 and 0.14, respectively; $P = 0.158$). The $\epsilon 3/3$ (76 and 78%) and $\epsilon 3/4$ (16 and 23%) were the most common genotypes for patients and controls, respectively. The lipid profile was altered in patients compared to controls ($P < 0.05$), independently of the $\epsilon 4$ allele. However, in the controls this allele was prevalent in individuals with elevated LDL-cholesterol levels only (odds ratio = 2.531; 95% CI = 1.028-6.232). The frequency of risk factors was higher in the CAD group ($P < 0.05$), but their association with the lipid profile was not demonstrable in $\epsilon 4$ carriers. In conclusion, the $\epsilon 4$ allele is not associated with CAD or lipid profile in patients with atherosclerosis. However, its frequency in the non-CAD group is associated with increased levels of LDL-cholesterol, suggesting an independent effect of the $\epsilon 4$ allele on lipid profile when the low frequency of other risk factors in this group is taken into account.

Key words

- Apolipoprotein E polymorphism
- Coronary artery disease
- Lipid profile

Correspondence

D.R.S. Souza
Departamento de Biologia Molecular
Faculdade de Medicina de São José
do Rio Preto
Av. Brigadeiro Faria Lima, 5416
15090-000 São José do Rio Preto, SP
Brasil
Fax: +55-11-3201-5700
E-mail: doroteia@famerp.br

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Introduction

Apolipoprotein E (apoE), identified at the beginning of the 1970's, has been associated with coronary artery disease (CAD), as well as other illnesses including cerebrovascular, peripheral artery and neurodegenera-

tive diseases such as late-onset Alzheimer's disease (1-8), as a result of the allelic variations of the *APOE* gene ($\epsilon 2$, $\epsilon 3$, and $\epsilon 4$) (3). ApoE polymorphisms regulate changes in lipid metabolism, platelet aggregation and processes of oxidative stress (9). However, their influence on serum lipids in patients

with CAD is of particular interest.

The apoE isoforms differ in their affinity for specific receptors including the low-density lipoprotein (LDL) receptor and LDL receptor-related protein. ApoE4 has a slightly greater affinity for these receptors compared to apoE3, while apoE2 rarely binds, having a binding capacity of less than 2% of that of apoE3 (10). Hence, apoE polymorphisms are related to the concentration of lipids and lipoproteins in the circulation of human populations, accounting for 4 to 15% of the variations in the serum levels of the LDL cholesterol fraction (LDLc) (6). The ϵ 4 allele (E3/4 and E4/4 phenotypes) has been associated with elevated serum LDLc levels and consequently it is considered to be a risk factor for CAD, while the ϵ 2 allele (E2/2 and E2/3) seems to be related to low levels of LDLc and increased concentrations of plasma triglycerides (TG) and lipoprotein remnants (3).

High LDLc and total cholesterol (TC) values were observed in decreasing order in individuals with phenotypes E2/3 < E3/3 < E4/3 < E4/4 (11). Moreover, the high frequency of the ϵ 4 allele associated with increased levels of TG and decreased high-density lipoprotein cholesterol (HDLc) levels suggested its role as one of the factors contributing to the high mortality rate from cardiovascular disease in Australian aborigines (12). Furthermore, a report stated that genotypes with the ϵ 2 and ϵ 4 alleles are more commonly seen at health centers that treat lipid-based diseases compared to the ϵ 3 allele (13). The ϵ 4 allele is also related to an increased risk of silent myocardial infarction induced by exercise in apparently healthy normolipidemic elderly men (14). A study of possible genetic markers in Brazilian women demonstrated an association of the apoE and apoB polymorphisms and those of the LDL receptor gene with CAD (4). However, in Chinese patients, the apoE allelic frequencies were similar in patients and controls (15). In Italians, the ϵ 4 allele was not correlated to an increase in risk for acute

myocardial infarction in patients with CAD (16).

Thus, the effect of the apoE polymorphisms on the serum lipid levels is variable. The ϵ 2 allele showed a positive correlation with lipid levels in an elderly multiethnic population, while the ϵ 4 allele was not expressed (17), and was not associated with changes in the lipid profile of a rural North American population (18). The extent of atherosclerotic injury in the coronary arteries and aorta and the levels of serum lipids also proved to be unaffected by the apoE genotypes in North Americans (19).

The objective of the present study was to evaluate the allelic and genotypic frequencies for apoE in Brazilian subjects with CAD and non-CAD and their influence on the lipid profile together with other risk factors for the disease.

Material and Methods

Patients

A total of 128 male (64%) and 72 female (36%) unrelated individuals of mixed ethnicity, representative of Brazilian population, ranging in age from 40 to 78 years were assessed in a case-control type study (20). The individuals were attended at the Hospital of São José do Rio Preto Medical School, São José do Rio Preto, SP, Brazil, and were divided into two groups: CAD group, 100 patients (60.8 ± 10.0 years) with lesions confirmed by coronary angiography, and non-CAD group, 100 individuals (60.1 ± 8.8 years; $P = 0.573$) with clinical signs suggestive of CAD and with an indication for coronary angiography during the diagnostic procedure but with proved absence of coronary obstruction.

The study was approved by the Research Ethics Committee of the São José do Rio Preto Medical School, SP, Brazil. All individuals signed written consent forms before being included in the study.

ApoE polymorphism, lipid profile and personal antecedents

The study of the apoE polymorphisms involved DNA extraction, amplification by the polymerase chain reaction, enzymatic digestion with *HhaI*, and 6% polyacrylamide gel electrophoresis with ethidium bromide staining (21).

The lipid profile was analyzed when patients were not taking lipid-reducing medications. Serum TG and TC levels were determined by enzymatic colorimetric methods (22). The serum levels of HDLc were analyzed by precipitation with dextran-magnesium chloride (23) and the Friedewald formula was used to calculate LDLc and VLDLc levels for TG of less than 400 mg/dL. The reference values used were those recommended by the III Brazilian Guidelines on Dyslipidemias and Guidelines of Atherosclerosis Prevention from the Atherosclerosis Department of Sociedade Brasileira de Cardiologia (24).

Both groups were evaluated with respect to the prevalence of hypertension, diabetes mellitus and smoking. Individuals were considered to be hypertensive if arterial blood pressures were equal to or higher than 140/90 mmHg for systolic and diastolic blood pressures, respectively, or if they were taking anti-hypertensive drugs. Diabetes mellitus was reported by patients diagnosed by a physician or whose laboratory examinations showed fasting blood sugar levels higher than 126 mg/dL (25). Smokers were those individuals who smoked at least one cigarette per day or who had stopped smoking within the previous two years. These risk factors were also studied considering the apoE polymorphisms and the lipid profile.

Statistical analysis

The allelic and genotypic frequencies, calculated by counting the alleles, were compared between groups using the Fisher exact

test or the chi-squared test, that was used also to compare the observed and expected genotypic frequencies in order to test Hardy-Weinberg equilibrium (HWE) and to compare the frequencies of hypertension, diabetes mellitus and smoking between groups. Differences in serum levels for the lipid profile were compared between groups by the *t*-test. The odds ratio was calculated as an estimate of the relative risk with a 95% confidence interval to assess the association between the $\epsilon 4$ allele and the lipid profile in patients and controls. $P < 0.05$ was considered to be significant.

Results

ApoE polymorphisms in CAD and non-CAD subjects

The $\epsilon 3$ allele was the most common in both the CAD (0.87) and non-CAD (0.81) groups, followed by the $\epsilon 4$ allele (0.09 and 0.14, respectively) and the $\epsilon 2$ allele (0.035 and 0.050, respectively, Table 1). No differences in allelic distribution were identified with respect to gender or age (<60 years and

Table 1. Allelic and genotypic frequencies of apolipoprotein E in patients with coronary artery disease (CAD group) and controls (non-CAD group).

Allele	CAD group		Non-CAD group	
	N	Frequency	N	Frequency
$\epsilon 2$	7	0.035	10	0.050
$\epsilon 3$	175	0.875	162	0.810
$\epsilon 4$	18	0.090	28	0.140
Total	200	1.000	200	1.000
Genotype	N	%	N	%
$\epsilon 2/\epsilon 2$	0	0	2	2
$\epsilon 2/\epsilon 3$	7	7	3	3
$\epsilon 2/\epsilon 4$	0	0	3	3
$\epsilon 3/\epsilon 3$	76	76	68	68
$\epsilon 3/\epsilon 4$	16	16	23	23
$\epsilon 4/\epsilon 4$	1	1	1	1
Total	100	100	100	100

N = number of individuals. There were no significant differences ($P > 0.05$, Fisher test).

≥60 years) in either group (data not shown). For patients and controls, the most common genotypes were ε3/ε3 (76 and 68%, respectively) and ε3/ε4 (16 and 23%, respectively) (Table 1). There were no differences in allelic or genotypic frequencies between groups. The calculation performed to test HWE showed that the genotypic distribution in the CAD group was similar to that expected ($\chi^2_3 = 0.927$; $0.80 < P < 0.90$). However, the non-CAD group showed departure from HWE, with a difference between the observed and expected genotypic proportions ($\chi^2_3 = 18.494$; $P < 0.001$).

Lipid profile and risk factors

There was a difference in the lipid profile of the CAD group compared to the non-CAD group (Table 2). The mean values for all the variables of the non-CAD group were within the recommended range, except for TG, and even then, with a lower mean value (169.4 ± 111.2 mg/dL) compared to the CAD group (230.5 ± 163.1 mg/dL; $P = 0.010$). The frequencies of hypertension, diabetes mellitus and smoking were 66, 29, and 59%, respectively, for the CAD group and 28, 10, and 40%, respectively, for the non-CAD group ($P < 0.05$).

Table 2. Lipid profile of patients with obstructive coronary artery disease (CAD group) and controls (non-CAD group).

Lipid profile (mg/dL)	CAD group (N = 100)	Non-CAD group (N = 100)
TC	226.5 ± 58.1	194.8 ± 51.1*
LDLc ^a	143.1 ± 40.2	113.4 ± 38.6*
HDLc	36.3 ± 12.1	52.7 ± 48.0*
VLDLc ^a	34.4 ± 16.9	30.6 ± 13.1
TG	230.5 ± 163.1	169.4 ± 111.2*

Data are reported as mean ± SD. TC = total cholesterol; LDLc = low-density lipoprotein cholesterol; HDLc = high-density lipoprotein cholesterol; VLDLc = very low-density lipoprotein cholesterol; TG = triglycerides. ^aN = 84 patients and 96 controls.

* $P < 0.05$ compared to the CAD group (Fisher test).

ApoE polymorphisms, lipid profile and risk factors

The association of the lipid profile with the apoE polymorphisms for the CAD and non-CAD groups observed by comparing genotypes with at least one ε4 allele (ε4/ε4 + ε3/ε4 + ε2/ε4) and those without the ε4 allele is shown in Figure 1. The variations in the lipid profiles within each group with respect to the presence or absence of the ε4 allele were similar. Nevertheless, the CAD group presented higher mean TC and LDLc values compared to the non-CAD group (recommended values) both with ($P = 0.023$ and 0.004 , respectively) and without the ε4 allele ($P = 0.007$ and 0.0001 , respectively). HDLc levels were lower in patients with the ε4 allele compared to the non-CAD group (35 ± 11 , 51 ± 24 mg/dL; $P = 0.025$), and the values also differed between groups in the absence of the ε4 allele ($P < 0.0001$). For VLDLc, no difference was observed between groups, with both presenting recommended levels in the presence or absence of the ε4 allele (data not shown). On the other hand, for TG levels, even though the mean value was higher in the CAD subgroup with the ε4 allele, a difference was detected between groups only for the genotypes without the ε4 allele ($P = 0.041$).

Figure 2 shows the effect of the ε4 allele on the CAD and non-CAD groups and the difference (in percentage) of the mean levels of TC, LDLc, HDLc, and TG between the subgroups with and without the ε4 allele. Note that in the CAD group the mean levels of TC (an increase of 22 mg/dL) and LDLc (an increase of 13 mg/dL) were about 10% higher in the subgroup with the ε4 allele, while in the non-CAD group the values were 3.1 and 4.3%, respectively (increases of 6 and 5 mg/dL, respectively). In the presence of the ε4 allele, a higher value of 16.3% (an increase of 37 mg/dL) was observed for TG in the CAD group, while in the non-CAD group there was a reduction of 2.2% (a drop

of 4 mg/dL). HDLc levels presented a reduction of 5% (a drop of 2 mg/dL) in patients with the ε4 allele and a 5.3% increase (an increase of 3 mg/dL) in the non-CAD group. The odds ratio showed an increase in LDLc

levels among individuals carrying the ε4 allele in the non-CAD group (odds ratio = 2.531; confidence interval = 1.028-6.232; Table 3).

An analysis of the apoE polymorphisms

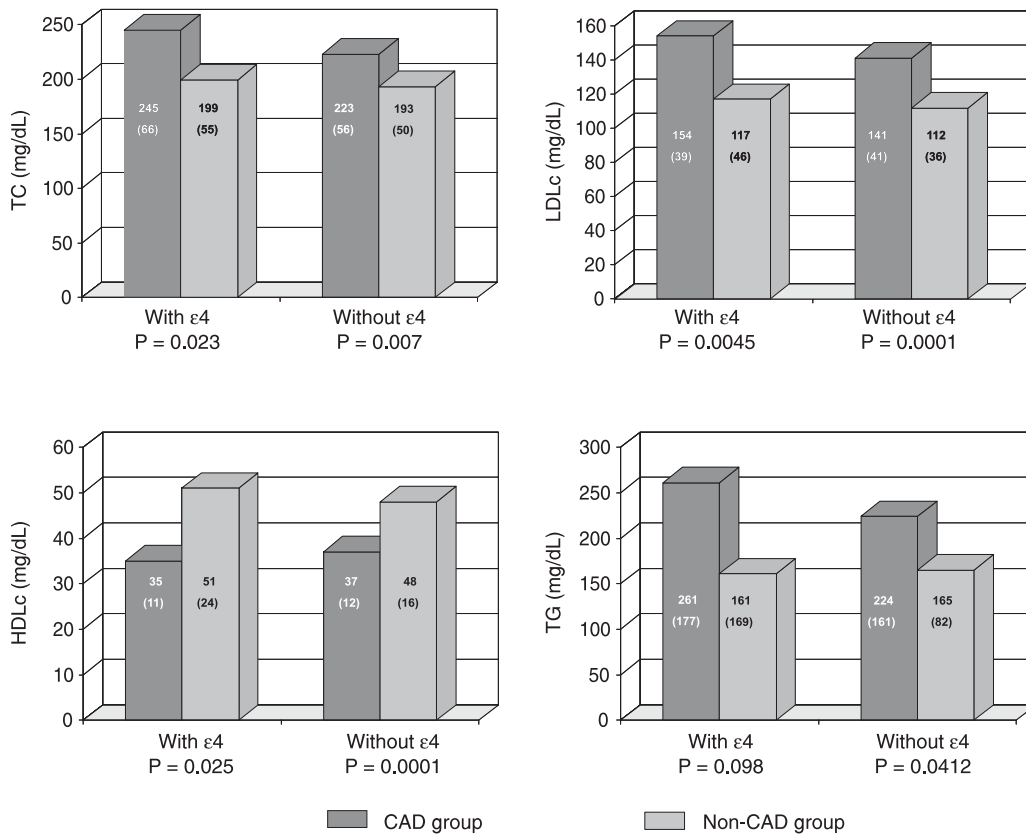


Figure 1. Total cholesterol (TC), low-density lipoprotein cholesterol (LDLc), high-density lipoprotein cholesterol (HDLc), and triglycerides (TG) in the presence or absence of the ε4 allele of apolipoprotein E in patients with coronary artery disease (CAD group) and controls (non-CAD group). Data are reported as means with SD reported in parentheses on each bar. Differences among the serum levels for the lipid profile between groups were not significant (P > 0.05, t-test).

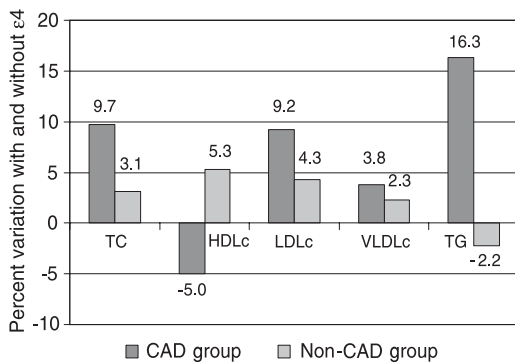


Figure 2. Effect of the ε4 allele of apolipoprotein E on the levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDLc), high-density lipoprotein cholesterol (HDLc), very low-density lipoprotein cholesterol (VLDLc), and triglycerides (TG), considering the percent variation of the lipid profile in relation to the presence or absence of the ε4 allele between patients with coronary artery disease (CAD group) and controls (non-CAD group).

associated with hypertension and diabetes mellitus did not show differences between the groups regarding the allelic and genotypic distributions. There was no association between risk factors and presence of the $\epsilon 4$ allele in either group or between this allele and increased LDLc levels in individuals with hypertension or diabetes mellitus. Also, no differences in sex or age were observed (data not shown).

Discussion

In the present study, the lower frequency of the $\epsilon 4$ allele in patients with CAD suggests the absence of its association with the disease (16-18) in Brazilian patients, contrary to other reports on specific populations (4,26). Moreover, the $\epsilon 4$ allele seems not to influence the lipid profile in the CAD group (24-26), although these individuals present reduced levels of HDLc and increased levels of TC, LDLc, and TG, but also higher frequencies of the risk factors. On the other hand, the non-CAD group, with reduced frequencies of hypertension, diabetes mellitus and smoking compared to the CAD group, showed higher levels of LDLc associated with the $\epsilon 4$ allele compared to the presence of $\epsilon 3$. This reinforces the influence of the $\epsilon 4$ allele on the lipid profile of the population, considering its action on the metabolism of serum lipids (3).

The genotypic distribution observed in the CAD group agreed with what was expected according to HWE. However, this did not occur in the non-CAD group. Other investigators have reported similar situations in case-control type studies with the analysis of single nucleotide polymorphisms, with a departure from HWE in patients or controls or in both groups (5,27). In this case, apart from possible evolutive factors, the sample size, possible systemic errors while genotyping, failures while applying or interpreting the test, and even the adopted genetic disease model (dominant, recessive, additive, and multiplicative) should be considered (27). In addition, genes with late-onset manifestations may be simultaneously associated with other lethal diseases of earlier onset; the $\epsilon 4$ allele of apoE is a known risk factor for acute myocardial infarction, stroke, and peripheral artery disease, among other diseases (1,3,8,14). Hence, there is a competitive risk of death distancing HWE from more elderly control individuals. Likewise, selection of elderly individuals in itself favors the grouping of subjects with the $\epsilon 2/\epsilon 2$ genotype in the non-CAD group, considering the protective effect of the $\epsilon 2$ allele against CAD (3,28).

In the present study, the allelic and genotypic distributions did not differentiate patients from controls. However, studies of younger populations, possibly under less in-

Table 3. Odds ratio for altered lipid profile according to the frequency of the $\epsilon 4$ allele for apolipoprotein E in patients with coronary artery disease (CAD group) and controls (non-CAD group).

Group	Odds ratio (CI)				
	TC	LDLc	HDLc	VLDLc	TG
CAD	2.039	2.276	0.8021	2.314	0.6042
95% CI	(0.6112-6.804)	(0.7524-6.884)	(0.2471-2.603)	(0.6521-8.213)	(0.2106-1.734)
Non-CAD	0.778	2.531*	0.3704	1.889	0.8462
95% CI	(0.3181-1.092)	(1.028-6.232)	(0.1114-1.231)	(0.7421-4.808)	(0.3296-2.173)

CI = confidence interval; TC = total cholesterol; LDLc = low-density lipoprotein cholesterol; HDLc = high-density lipoprotein cholesterol; VLDLc = very low-density lipoprotein cholesterol; TG = triglycerides. *Higher frequency of increased LDLc levels in the non-CAD group (P = 0.0488).

fluence of environmental factors, might more reliably demonstrate the effect of the apoE polymorphisms. Salazar et al. (4), in a study of Brazilian subjects, detected an association between CAD and the $\epsilon 4$ allele. However, contrary to the current study, younger non-diabetic women (48 ± 9 years) were studied, with a frequency of 0.07 for the $\epsilon 2$ allele, 0.70 for $\epsilon 3$, and 0.23 for $\epsilon 4$. Also a study on a Finnish population showed elevated levels of the $\epsilon 4$ allele (0.32) associated with a higher risk of CAD (29). Moreover, Cumming and Robertson (6) reported in a Scottish population a lower mean age at the first infarction in men with the $\epsilon 3/\epsilon 4$ genotype compared to the other genotypes. In contrast, Lenzen et al. (30) did not observe any difference in the apoE alleles and genotypes between survivors of acute myocardial infarction and a control group. Furthermore, studies of different populations including Chinese (15) and Italians (16) support the lack of association between the $\epsilon 4$ allele and CAD.

Recently, a meta-analysis of apoE genotypes and the risk for CAD was performed, including 48 studies published from 1983 to 2003 (15,492 cases and 32,965 controls) mainly of European, North American and Asian populations suffering non-fatal or fatal myocardial infarctions and angiographically confirmed CAD. The study demonstrated a higher risk (42%) for CAD in individuals with the $\epsilon 4$ allele compared to those with the $\epsilon 3/\epsilon 3$ genotype (5). The results are consistent with those reported previously by another meta-analysis that analyzed 14 studies (3087 cases and 7059 controls) including clinically or angiographically diagnosed CAD (7). These systematic reviews stated that inadequate statistical analysis, geographic and ethnic differences, gender, CAD, end points, study design, and potential gene-environment interactions can contribute to the differences among authors.

There is also controversy with respect to the association of apoE genetic polymor-

phisms with changes in the lipid profile of individuals with CAD. In the current study, this association was not confirmed even when only lipid and lipoprotein levels higher than borderline were considered (data not shown). Scuteri et al. (31), evaluating the $\epsilon 4$ allele as an independent risk factor for coronary artery events in men only, did not observe this association with increased TC levels. Additionally, in Japanese individuals, although the $\epsilon 4$ allele was two times more common in CAD patients (0.23) compared to controls (0.10), there was no impact of its effect on LDLc levels identifying it as an independent risk factor for the disease (32).

On the other hand, although not many, there are reports that the apoE alleles are genetic markers for dyslipidemia and CAD (33). Studies have estimated the impact of apoE polymorphisms on the levels of cholesterol and on the risk for CAD, showing that 7% of the variation in cholesterol levels is associated with the apoE gene but only 2.8% of the variation can be considered to represent a risk for CAD, corresponding to a considerable contribution for a single genetic factor (3).

In the present study, patients with at least one $\epsilon 4$ allele demonstrated a 10% increase in TC and LDLc levels and a 16% increase in TG levels. Corbo et al. (34) also did not observe an association of the $\epsilon 4$ allele with increased LDLc levels in Italian individuals. However, male patients showed higher levels of TC and LDLc, in particular those with the $\epsilon 3/\epsilon 4$ genotype compared to those with the $\epsilon 3/\epsilon 3$ and $\epsilon 3/\epsilon 2$ genotypes. In this case the particularities of the genetic and environmental factors of the population, as well as the age (35), were highlighted as possibly influencing the effect of the apoE genotypes on the lipid profile.

Lower cholesterol levels have been the target of secondary atherosclerosis prevention programs. In this way, an increase in serum lipid levels in $\epsilon 4$ allele carriers, even if insignificant, might contribute to a less

favorable clinical evolution compared to the patients with recommended cholesterol levels. Incidentally, studies with therapeutic interventions have demonstrated the benefit of reducing serum cholesterol levels. In these studies, a 10% reduction in TC levels reduced the risk of non-fatal myocardial infarction by 19%, the risk of fatal infarction by 12%, and both by 15% (36). Moreover, this disease may be aggravated by other risk factors related to the development of the atherosclerotic process such as hypertension, diabetes mellitus and smoking (24,37), which in the present study were more common in the CAD group than in non-CAD group, although there was no association with apoE polymorphisms.

Mechanisms have been proposed to try to explain the association between apoE and atherosclerosis. In this way, it was recently reported that the action of apoE depends on gender and type of allele, on reverse cholesterol transport, on platelet aggregation, and on oxidative processes. These probably affect the overall atherogenic potential due to modulation of lipoprotein metabolism (9). Surprisingly, in an experiment with animals Raffai et al. (38) demonstrated the capacity of apoE to cause regression of atherosclerosis

independent of serum cholesterol levels. In this case, it is possible that a class of HDL-containing apoE promotes the removal of cholesterol from the foam cells at the injury site. Moreover, endothelial dysfunction, also present, may be reversed by the high serum level of apoE associated with an increase in the production of nitric oxide in the arterial wall (38).

It is evident that the predisposition to a complex disease such as CAD is determined by multiple genetic and environmental factors, with the possibility that one gene locus could identify a subgroup of at-risk individuals being unlikely. Thus, the synergic effect between genetic polymorphisms might explain the variations in the lipid profile, including the association of apoE with angiotensinogen, apoB, LDL receptor, apoB signal peptide, and lipoprotein lipase (39,40).

In this study the $\epsilon 4$ allele did not act as a risk factor for CAD. Furthermore, variations in the lipid profile of patients seem to be independent of apoE polymorphisms. However, in the non-CAD group, the increased LDLc levels are associated with the $\epsilon 4$ allele, while the frequency of other risk factors is reduced.

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