Apolipoprotein polymorphism is associated with pro-thrombotic profile in non-demented dyslipidemic subjects

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Abstract

Apolipoprotein gene polymorphism has an important role in lipid metabolism and in the development of cerebro- and cardio-vascular disease (CCVD), including dementia. Dyslipidemia and hemostatic abnormalities are key risk factors associated with athero-sclerotic events preceding CCVD. The aim of this study was to evaluate the possible relationships of various apolipoprotein-species with hemostatic parameters and cognitive function. Lipid profile, gene polymorphism, coagulation markers, and mini-mental state examination (MMSE) scores were assessed in 109 dys-lipidemic subjects and in 107 healthy control volunteers. Thrombin-activatable fibrinolysis inhibitor (TAFI) plasma levels were significantly higher in apolipoprotein-E2 (apoE2) patients when compared to other apoE forms. The apoA5 -1131T>C polymorphism was associated with elevated D-dimer concentration in dyslipidemic TT homozygous individuals. MMSE did not correlate with lipid or coagulation profile. These data suggest that apoE and apoA5 variants have an effect on hemostatic parameters, but they neither influence nor predict cognitive performance in non-demented individuals.

Keywords: Apolipoprotein-A5 (apoA5), apolipoprotein-E (apoE), cerebro- and cardio-vascular disease (CCVD), cognitive performance, D-dimer, dementia, dyslipidemia, gene polymorphism, thrombin-activatable fibrinolysis inhibitor (TAFI)

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Introduction

Cerebro- and cardio-vascular diseases (CCVD) are the leading cause of mortality and morbidity in industrialized countries, and increased risk for CCVD could not be solely explained with many of traditional, Framingham-type risk factor.^{1–4} Dyslipidemia is an important risk factor associated with atherosclerotic events preceding CCVD.^{4–6} Atherosclerosis is multifactorial disorder, in which an altered lipid metabolism, activated endothelial and the coagulation systems, and hypofibrinolysis play predominant roles. The development of atherosclerotic lesions is markedly promoted by interactions of plasma lipids, endothelium, platelets, coagulation factors and fibrinolytic system with the affected arterial vessel wall.^{7–9} Several studies have reported associations between lipids and hemostatic factors which could lead to disturbances in coagulation, and dyslipidemia, and hemostatic abnormalities has been shown to be associated with increased risk of CCVD and venous thrombo-embolism.^{1,10–16}

A possible pathway linking dyslipidemia and cardiovascular risk could be through associations with hemostatic

factors.¹⁷ Elevated levels of thrombomodulin (TM) and homocysteine (HCY) have been reported in conditions related to endothelial cell damage such as hyperlipidemia, diabetes, hypertension and vascular disease.^{10,11,18} Prospective studies have reported increased risk of cardio-vascular events and dementia in patients with coagulation activation or impaired fibrinolytic function.^{16,19} In fact, high levels of D-dimer and prothrombin fragment 1+2 (F1+2), which indicate a global coagulation activation, have been reported in patients with recurrent thrombotic events, the highest risk of developing venous thromboembolism,²⁰ cognitive decline,¹⁶ in aortic stenosis and myocardial infarction patients.^{14,15} Consistent findings have been reported regarding increased levels of plasminogen activator inhibitor 1 (PAI-1) and thrombin-activatable fibrinolysis inhibitor (TAFI), associated with total cholesterol and low density lipoproteins (LDL), triglycerides, patients with acute coronary syndromes, venous thrombo-embolism and with an increased incidence of cardiovascular events.^{1,13,14,21–23}

It is well known that dyslipidemia is a complex trait caused by multiple environmental and genetic factors and their interactions.^{24,25} The derangements of lipoprotein

metabolism are characterized by apolipoproteins (apo) abnormalities rather than lipid profile. Lipid profile is also in part determined by genetic variations in apo's. Characterization of the molecular variability of candidate genes could allow identification of population at cardiovascular risk.²

Apolipoprotein-E (apoE) and apolipoprotein-A5 (apoA5) belong to the key proteins involved in the lipoprotein metabolism and CCVD.^{4,26–28} apoE is a component of chylomicrons, very low density lipoproteins (VLDL), remnant particles, and high density lipoproteins (HDL).¹³ The structural gene for apoE is polymorphic. Three common alleles designated $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$ code for protein isoforms E2, E3, and E4.^{27,29} Associations among this polymorphism, plasma lipid and lipoprotein levels, and atherosclerosis, have been well documented.^{26,27,30} In addition, the role of apoE alleles and isoforms in the etiology and pathogenesis of Alzheimer's disease (AD) has also been recently discussed by several authors.^{31–34} The apoA5 was found predominantly bound to VLDL and HDL in the plasma.^{35,36} Several groups have investigated the mechanisms underlying effects of apoA5 on Tg metabolism.^{37,38} The presence of naturally existing polymorphisms can modify the effect of apoA5 on the lipid metabolism, which can result in increased TG concentrations.³⁸ The apoA5 can be involved in the abnormal accumulation of lipids in the endothelial cells under pathologic conditions, ultimately in the formation of atherogenic plaques that are key elements in obstructive vascular disease.²⁸

Although dyslipidemia often occurs in patients with cardiovascular disease, the underlying mechanisms remain poorly understood.²⁸ The association between hemostatic dysfunction and atherosclerosis is also largely unclear.³⁹ A major focus of recent research in CCVD has been to understand the molecular basis of atherosclerosis and the identification of the role of apolipoproteins in this process.^{4,40} Previous genetic studies have demonstrated that variants apoE and apoA5 are significant determinants of serum lipid levels in dyslipidemic subjects and CCVD. However, only a few studies have investigated the association of these variants with hemostatic parameters.^{1,41} Therefore, the aim of this study was to evaluate the possible relationships between apoE and apoA5 genes polymorphisms and hemostatics parameters in dyslipidemics subjects and healthy volunteers.

Methods

Subjects

The study included 216 individuals, aged from 30 to 60 years, all of whom were recruited from Socor Hospital, Santa Casa Hospital and Petrobras-Gabriel Passos Refinery in the state of Minas Gerais in Brazil. Participants were divided into two groups, including 109 dyslipidemic subjects (41 women and 68 men, with a mean age of 48.4 ± 6.8 years) and 107 normo-lipidemic healthy volunteers (63 women and 44 men; mean age: 46.7 ± 6.6 years). Lipid levels were classified according to the National Cholesterol Education Program.⁴² Participants were thus diagnosed

with dyslipidemia if they had one or more of the following four components: (1) a serum concentration of total cholesterol (Tc) of ≥ 6.24 mmol/L; (2) a serum concentration of (Tg) triglycerides ≥ 2.26 mmol/L; (3) a serum concentration of high density lipoprotein (HDL)-cholesterol (HDLc) of < 1.04 mmol/L for men or < 1.30 mmol/L for women; and (4) a serum concentration of low density lipoprotein cholesterol (LDLc) ≥ 4.14 mmol/L. Cognitive function was measured by mini-mental state examination (MMSE); a score above 27 was considered normal (lack of cognitive impairment). Pseudo-dementia of depression was ruled out by Beck depression inventory. The exclusion criteria included diabetes mellitus, metabolic syndrome, hypo-thyroidism, renal-, hepatic- and auto-immune diseases, subjects taking lipid-lowering agents, oral anticoagulants and/or anti-inflammatory drugs. A complete medical check-up and history for cardio/cerebro-vascular diseases was obtained, including routine biochemical studies to exclude any systemic disorders. Detailed information on cardio-vascular risk factors, including hypertension, smoking, alcohol consumption, and use of drugs was obtained from each individual. Hypertension was defined as blood pressure being $\geq 140/90$ mmHg measured at two different occasions and/or the use of anti-hypertensive medication. Those individuals who did not practice physical exercises regularly and for at least 40 minutes, three times a week were considered sedentary.⁴³ Ethics board approval was obtained by the local ethical committee for this study. All participants signed an informed consent, and a clinical report was completed.

Methods

Venous blood samples were drawn after 12–14 h overnight fast. Blood samples were immediately centrifuged at 2500 rpm for 20 min at 4°C, and the supernatant was collected and stored at -80°C until measurement. Plasma Tc, Tg, and HDLc levels were measured enzymatically (Bioclin®). LDLc was calculated according to the Friedewald equation⁴⁴ except for patients with $\text{Tg} > 4.5$ mmol/L. Apolipoprotein-A-I (apoA-I), apolipoprotein-B (apoB), and lipoprotein-A (LpA) were measured by immuno-turbidimetry assays (Biotécnica). Plasma concentrations of F1+2, D-dimer, PAI-1, and TAFI were determined with the enzyme immunoassay ENZYGNOST F1+2 (monoclonal) (Dade Behring Marburg GmbH, Germany), Imubind® D-dimer ELISA (American Diagnostics, Stamford, USA), Imubind® Plasma PAI-1 ELISA (American Diagnostics, Stamford, USA), and Visulize™ TAFI antigen kit (Affinity Biologicals, Canada), respectively. Homocysteine was measured by chemiluminescence assay (Advia Centaur® Homocysteine, Bayer HealthCare, Tarrytown, NY, USA). apoE and apoA5 polymorphisms were genotyped as described previously.^{4,28}

Statistical analysis

Data were tested for normality. Tg and Tc data were log-transformed to obtain normal distribution. All values are expressed as mean $\pm$ standard deviation (SD). Comparison of means among groups was calculated by analyses of

variance (ANOVA). Statistically significant differences among groups were evaluated by Student-Newman-Keuls (SNK) test. To examine the association between apoE genotypes, cognitive function and hemostatic parameters, the individuals were distributed into three sub-groups: apoE2 sub-group (ε2ε3 individuals), apoE3 sub-group (ε3ε3 subjects), and apoE4 sub-group (ε3ε4 and ε4ε4 persons). The ε2ε2 and ε2ε4 genotypes were not detected in either sub-group. The genotypes for the apoA5 -1131T>C gene were grouped as C-carriers (TC+CC) and non-carriers (TT) for analysis with hemostatic parameters and cognitive function. To investigate a possible association among risk factors, apoE and apoA5 alleles, hemostatic parameters, cognitive function, and dyslipidemia, the model of analysis logistic multivariate regression was performed. The *P* value of less than 0.05 was considered to be statistically significant. All statistical analyses were done using SAS 9.0, SAEG 8.1, and Sigma Stat 2.0 softwares.

Results

Characterization of the studied groups

The demographic characteristics, lipid-lipoprotein, and apolipoproteins plasma levels in the normo-lipidic and dyslipidemic groups have been described previously⁴. There was no significant age difference between the groups, but the presence of women was higher in the normo-lipidic group and men in the dyslipidemic group. As expected, Tc, LDLc, Tg, and non-HDLc levels, as well as apoB/apoA-I ratio were higher in dyslipidemic subjects compared with normo-lipidic participants (*P* < 0.0001). HDLc and apoA-I plasma levels were higher in the normo-lipidic group, when compared to that seen with dyslipidemic patients (*P* < 0.0001). No significant differences were observed for apoB and LpA levels between the groups.

With respect to clinical characteristics, no differences have been found among the frequencies of smoking, sedentary lifestyle, and alcohol consumption between the groups (dyslipidemic: 18.4, 50.5, and 6.4%; normo-lipidic: 16.8, 48.6, and 1.9%, respectively), as well for oral contraceptive use and hormonal therapy between women in both groups. The dyslipidemic subjects presented higher frequency of hypertension (*P* = 0.0001), body mass index (BMI, *P* = 0.001) in the obese range and of women in menopause (*P* = 0.001), when compared to normo-lipidic individuals. Blood pressure lowering drugs were used by many participants reflecting the high prevalence of hypertension, especially in the dyslipidemic group. There was no statistically significant difference between groups with respect to their higher cerebral performance, and none of the participants had cognitive impairment or dementia (MMSE: 28.1 ± 0.8 *vs.* 28.3 ± 1.5). These variables were not significantly different between case and control groups when adjusted according to gender (data not shown). Given the extensive miscegenation of the volunteers, the ethnicity of each participant was not defined.⁴⁵

Hemostatic parameters between sub-groups E2, E3 and E4 in apoE polymorphism

The results of hemostatic parameters between groups, alleles and genotypes frequencies apoE and apoA5 were previously described.^{4,28,46,47}

In order to analyze the effect of apoE polymorphism on hemostatic parameters in dyslipidemic and normo-lipemic groups, individuals were divided into three apoE sub-groups: E2, E3, and E4 (Table 1). No significant differences were observed among E2, E3, and E4 and hemostatic plasma levels in the normo-lipemic group. However, in the dyslipidemic group, TAFI plasma levels were significantly higher in E2 patients compared to E4 (*P* < 0.05), but no significant differences were observed between E2 and E3 or E3 and E4 samples with respect to TAFI.

When data were analyzed distributing all participants in the three apoE sub-groups E2, E3, and E4, no significant difference was observed in the mean hemostatic parameters (Table 2). There was no statistically significant difference between apoE sub-groups with respect to their cognitive function (data not shown).

Hemostatic parameters between sub-groups C-carriers (TC + CC) and non-carriers (TT) in apoA5 -1131T>C polymorphism

When the association between apoA5 -1131T>C polymorphism and hemostatic parameters was evaluated separately in dyslipidemic and normo-lipidic groups, D-dimer concentration was higher in dyslipidemic TT homozygous than C-carriers (*P* = 0.016) (Table 3). However, when this analysis was performed with all participants, no significant differences for hemostatic parameters concentrations were observed in all groups, according to presence of the allele C (Table 4). There was no statistically significant difference between apoA5 C-carriers and non-carriers with respect to their cognitive function (data not shown).

Multivariate logistic regression

Multivariate logistic regression was performed with the group's dyslipidemic and normo-lipidic analyzed together. The logistic multivariate regression, considering the apoE and apoA5 alleles, hemostatic markers, cognitive performance, and the cardiovascular risk factors as independent variables, showed that all of these variables were not independently associated with dyslipidemia (data not shown).

Discussion

Atherosclerosis is increasingly recognized as a complex phenomenon involving the interaction of several mechanisms.^{9,48} Platelet activation, aggregation, and thrombosis play pivotal roles in atherosclerotic plaque formation, and progression and plaque rupture are accepted as the common pathogenetic pathways of thrombotic events. Many environmental and genetic factors play a role in its development. Abnormalities in lipid metabolism and coagulation system are the well-known risk factors for CCVD.^{16,19,26,49} Therefore, much effort has been devoted to discovering and understanding genetic mechanisms regulating the

Table 1 Hemostatic parameters in the apoE sub-groups E2, E3, and E4

		apoE sub-groups			P
Groups		E2 (n = 19)	E3 (n = 149)	E4 (n = 48)	
HCY*	Normo-lipidic	11.3 ± 2.9	11.1 ± 2.5	11.3 ± 2.5	0.929
	Dyslipidemic	11.5 ± 2.3	11.5 ± 3.5	10.6 ± 2.6	0.477
TM†	Normo-lipidic	3.1 ± 0.8	3.2 ± 1.0	2.8 ± 0.8	0.103
	Dyslipidemic	3.2 ± 0.7	3.2 ± 0.8	3.2 ± 0.8	0.990
F1 + 2‡	Normo-lipidic	211.0 ± 129.5	218.6 ± 114.2	176.9 ± 63.5	0.123
	Dyslipidemic	188.5 ± 63.4	222.5 ± 87.2	203.4 ± 88.5	0.387
D-Di†	Normo-lipidic	263.9 ± 128.6	262.5 ± 106.1	265.8 ± 184.1	0.814
	Dyslipidemic	232.3 ± 152.3	235.6 ± 118.0	215.0 ± 112.1	0.870
PAI-1†	Normo-lipidic	67.4 ± 16.9	54.9 ± 26.8	50.9 ± 25.0	0.154
	Dyslipidemic	50.3 ± 23.9	73.9 ± 123.8	58.4 ± 28.1	0.614
TAFI§	Normo-lipidic	7.3 ± 1.3	7.5 ± 1.5	7.2 ± 1.6	0.646
	Dyslipidemic	9.5 ^a ± 2.1	9.0 ^{ab} ± 1.6	8.3 ^b ± 1.0	<0.05

Note: Data are presented as mean ± SD, normo-lipidic (n = 107) and dyslipidemic (n = 109).

E2: ε2ε3 genotype (normo-lipidic n = 9, dyslipidemic n = 10), E3: ε3ε3 genotype (normo-lipidic n = 72 and dyslipidemic n = 77), E4: ε3ε4 and ε4ε4 genotypes (normo-lipidic n = 26, dyslipidemic n = 22). P < 0.05: statistically significant difference (the P values were estimated ANOVA followed by Student-Newman-Keuls test).

*Values are expressed as μmol/L.

†Represents concentrations in ng/mL.

‡Denotes levels in pmol/L.

§Expressed as μg/mL.

^aP > 0.05 (E2 vs. E3).

^bP > 0.05 (E3 vs. E4).

^{ab}P < 0.05 (E2 vs. E4).

Table 2 Hemostatic parameters in the apoE sub-groups

	E2 (n = 19)	E3 (n = 149)	E4 (n = 48)	P
HCY*	11.4 ± 2.5	11.3 ± 3.0	11.0 ± 2.5	0.740
TM†	3.2 ± 0.8	3.2 ± 0.9	3.0 ± 0.8	0.188
F1 + 2‡	199.2 ± 97.9	220.6 ± 100.8	189.1 ± 76.3	0.059
D-Di†	247.3 ± 138.6	248.6 ± 112.9	242.5 ± 155.9	0.779
PAI-1†	58.4 ± 22.2	64.7 ± 91.2	54.4 ± 26.4	0.669
TAFI§	8.5 ± 2.1	8.3 ± 1.7	7.7 ± 1.5	0.094

Note: Data are presented as mean ± SD. E2: ε2ε3 genotype, E3: ε3ε3 genotype, E4: ε3ε4 and ε4ε4 genotypes. P < 0.05: statistically significant difference (the P values were estimated ANOVA followed by Student-Newman-Keuls test).

*Values are expressed as μmol/L.

†Represents concentrations in ng/mL.

‡Denotes levels in pmol/L.

§Expressed as μg/mL.

hemostatic system and atherosclerosis, but data on the relationship between atherosclerotic plaque and genes involved in the lipids and lipoproteins metabolism that influence the coagulation system are sparse. Further investigations in this field may lead to novel treatments for both atherosclerosis and thrombosis.⁵⁰ Moreover, the association of the many genes thought to be involved in lipid and lipoprotein metabolism, coagulation system and CCVD remains unclear and their interaction is complicated by important non-genetic factors.⁵⁰

The present study was conducted to investigate markers of endothelial dysfunction (HCY and TM), impaired fibrinolysis (PAI-1 and TAFI) and hypercoagulability (F1+2 and D-dimer) in dyslipidemic subjects with apoE and apoA5 polymorphisms associated with dyslipidemia, atherosclerosis, and CCVD.

One of the major aims of this study was to investigate any relationship between apoE gene polymorphism and plasma levels of hemostatic parameters. In our study, we find association only between apoE genotypes and plasma TAFI levels. TAFI plasma levels were significantly higher in E2 patients compared to E4 dyslipidemics. apoE genotype is associated with elevated serum lipid levels and higher plasma PAI-1 concentrations.^{2,51,52} Similarly to our results, Mermod and colleagues demonstrated that there is no association between apoE genotype and circulating PAI-1 levels in healthy, resting individuals.⁵¹ It is noteworthy that this study was limited to a middle-aged population. Since PAI-1 plasma levels increase with age and since age is a major risk factor for CCVD, it may be important to

Table 3 apoA5 -1131T>C polymorphism and hemostatic parameters of the normo-lipidic and dyslipidemic groups

		apoA5 genotypes		P
groups		TT	TC + CC	
HCY*	normo-lipidic	11.2 ± 2.5	11.0 ± 2.4	0.642
	dyslipidemic	11.7 ± 3.7	11.0 ± 2.7	0.256
TM†	normo-lipidic	3.2 ± 1.1	3.0 ± 0.7	0.499
	dyslipidemic	3.2 ± 0.8	3.3 ± 0.8	0.665
F1 + 2‡	normo-lipidic	203.4 ± 87.5	216.6 ± 136.6	0.688
	dyslipidemic	226.0 ± 99.9	205.4 ± 70.3	0.440
D-Di†	normo-lipidic	249.5 ± 112.4	290.8 ± 156.2	0.142
	dyslipidemic	254.6 ± 128.9	205.1 ± 101.6	0.016
PAI-1†	normo-lipidic	55.0 ± 23.8	55.1 ± 29.9	0.634
	dyslipidemic	78.3 ± 149.7	59.9 ± 27.8	0.951
TAFI§	normo-lipidic	7.5 ± 1.4	7.2 ± 1.7	0.302
	dyslipidemic	9.0 ± 1.6	8.7 ± 1.6	0.375

Note: Data are presented as mean ± SD. normo-lipidic (n = 107) and dyslipidemic (n = 109).

TT genotype (normo-lipidic n = 71, dyslipidemic n = 52), TC and CC genotypes (normo-lipidic n = 36 and dyslipidemic n = 56). P < 0.05: statistically significant difference (the P values were estimated ANOVA followed by Student-Newman-Keuls test).

*Values are expressed as µmol/L.

†Represents concentrations in ng/mL.

‡Denotes levels in pmol/L.

§Expressed as µg/mL.

Table 4 apoA5 -1131T>C polymorphism and hemostatic parameters for all participants

	TT genotype (n = 123)	TC+CC genotypes (n = 92)	P
HCY*	11.4 ± 3.1	11.0 ± 2.6	0.273
TM†	3.2 ± 1.0	3.2 ± 0.8	0.970
F1 + 2‡	213.0 ± 93.2	209.8 ± 101.0	0.909
D-Di†	251.7 ± 119.2	238.7 ± 131.9	0.188
PAI-1†	64.9 ± 99.1	58.0 ± 28.7	0.887
TAFI§	8.2 ± 1.6	8.1 ± 1.8	0.961

Note: Data are presented as mean ± SD. All participants were grouped (n = 215) and apoA5 -1131T>C gene were grouped as C-carriers (TC+CC) and non-carriers (TT). P < 0.05: statistically significant difference (the P values were estimated ANOVA followed by Student-Newman-Keuls test).

*Values are expressed as µmol/L.

†Represents concentrations in ng/mL.

‡Denotes levels in pmol/L.

§Expressed as µg/mL.

examine the influence of the apoE genotype on the plasma level in older donors.^{51,52} In dialysis patient, this association was not demonstrated.²

Differently, Aldamiz-Echevarria et al. demonstrated that plasma PAI-1 activity was significantly higher in children with renal transplant with ε3ε4 genotype than in patients with ε3ε3 genotype.¹ In another study that included 198 patients with diabetic nephropathy and 192 patients with diabetes mellitus and microalbuminuria, higher PAI-1 levels were found in only male patients who had coronary heart disease and apoE3/E4 phenotype.⁵³

The significant influence of apoE genotype on plasma PAI-1 activity has not been recognized previously. Experimental data indicate that VLDL induces PAI-1 synthesis through a signaling pathway involving protein kinase

C-mediated mitogen-activated protein kinase activation.^{1,54} apoE is a key ligand that mediates liver uptake of both LDL and VLDL by the LDL receptor. Of the three isoforms (E2, E3, and E4), lipid particles containing apoE4 isoforms show an accelerated clearance in relation to lipid particles containing apoE2 isoforms, while E3 isoforms show intermediate values, thus determining differences in plasma LDL-cholesterol levels.¹ It is possible that a similar mechanism may also determine differences in plasma PAI-1. It is of great interest that the LDL receptor-related protein (LRP), a member of the LDL receptor gene family that acts as a multifunctional scavenger and signaling receptor, has ligands for both apoE and PAI-1, and thus participates both in lipid metabolism and in the homeostasis of proteinases and proteinase inhibitors.^{1,55}

TAFI, also known as carboxypeptidase R, carboxypeptidase U, plasma procarboxypeptidase B, and thrombin activatable procarboxypeptidase B, is a 60 kDa glycoprotein synthesized by the liver that circulates as a zymogen. The thrombin/thrombomodulin complex activates TAFI to activated TAFI (TAFIa), which can modulate the rate of fibrin degradation by removing C-terminal lysine residues that are essential for optimal activation of plasminogen by its physiologic activator, tissue plasminogen activator. Low fibrinolytic function due to high circulating levels or enhanced activity of TAFIa has been associated with hypercoagulable states and increased intravascular events.¹³ We show here the first report of variations in plasma TAFI levels in dyslipidemic associated with apoE polymorphism. Patients with the $\epsilon 2\epsilon 3$ genotype, who are known to have a defective binding to the apoB/E receptor, displayed 15% higher TAFI levels than $\epsilon 3\epsilon 4$ and $\epsilon 4\epsilon 4$ patients. Consequently, the effect(s) of apoE genotypes on TAFI levels may influence the fibrinolytic system increasing the risk of developing atherosclerosis and CCVD. The influence of different apoE genotypes in this process remains unknown. Thus, further studies are needed to confirm this finding in different populations and with a larger number of participants.

When the association between apoA5 -1131T>C polymorphism and hemostatic parameters was evaluated separately in dyslipidemic and normo-lipidic groups, D-dimer concentration was higher in dyslipidemic TT homozygous than C-carriers. However, when this analysis was performed with all participants, no significant differences for hemostatic parameters concentrations was observed in all group, according to presence of the allele C.

The apoA5 -1131T>C polymorphism has been reported to be associated with the risk of CCVD.⁵⁶ This association has been shown to be mediated by Tg levels in human studies.⁵⁷ Bi et al. found that CC homozygotes had approximately a twofold coronary artery disease risk compared with subjects with the TT genotype.⁵⁸ In a recent study, Jang et al. reported that the homozygosity of the -1131C allele was associated with 47% higher Tg as compared with TT subjects in Korean CAD patients.⁵⁹ Similarly, our findings show an association between apoA5 -1131T>C polymorphism and lipid concentrations when they were evaluated, and Tc and Tg concentrations were higher in C-carriers than TT homozygous.²⁸ The -1131T>C polymorphism is located in the promoter region of the apoA5 gene and there is no transcription factor binding sites identified in this location. Therefore, it is justified that the -1131T>C variant may not be functional leading to increased levels of Tg. However, -1131C may affect the transcriptional activity of the apoA5 gene.⁵⁶

Recently, Gaunt et al. suggested that the levels of a clotting factor may reflect the direct effect of variation on the gene for that factor, or the indirect effect of variation on different gene acting via a particular biochemical pathway. The latter offers scope for a single genetic variant to influence multiple coagulation traits downstream of that biochemical pathway.⁶⁰ One such pathway is triglyceride metabolism: triglyceride levels associate with Factor VII and other vitamin K-dependent clotting factor levels, and with elevated plasma viscosity. A genetic analysis of the

pleiotropic role of triglyceride-associated single nucleotide polymorphisms (SNPs) in coagulation phenotypes therefore offers scope to explore the potential 'causal' role of triglycerides in this system.⁶⁰

Gaunt et al.⁶⁰ identified several previously unreported associations and found that SNP in *ZNF259-apoA5* appear to have a pleiotropic effect on coagulation phenotypes. The *ZNF259-APOA5* locus contains *apoA5*, *apoA4*, *apoC3*, and *apoA1*, and has been robustly demonstrated to be associated with triglyceride levels.^{28,57,58,59} The relationship between triglyceride levels and coagulation measures is well established, with associations between hyperlipidemia and hypercoagulability demonstrated in both animal models and humans.⁶⁰⁻⁶² Gaunt and colleagues demonstrated an association between *ZNF259-apoA5* and plasma viscosity that may therefore be caused by the influence of genetic variants in these regions on triglyceride levels.⁶⁰ However, the analysis of SNPs associated with triglyceride levels and multiple coagulation traits may support an important role of triglyceride levels in different phenotypes.

The main limitations of this study were the lack of existence of an imaging test in our participants to verify the development of the atherosclerotic process and limited sample size. However, the results still provide potentially important functional information about the molecular basis of coagulation that could be important in the contexts of drug development or rare variant effect prediction. In either of these cases, a larger effect through the same molecular mechanism could be clinically important; hence, identifying the mechanism adds important information to the field.

In conclusion, apoE polymorphism significantly affects plasma level of TAFI in dyslipidemic subjects, suggesting that $\epsilon 2$ allele carriers may be more susceptible to development of atherosclerosis. Also, as serum D-dimer concentration is increased in carriers of the TT genotype, apoA5 could play a role in the pathogenesis of atherosclerosis. The results further show the need for prospective studies in dyslipidemic and normolipidemic to elucidate the impact of genetic variation at the apoE, apoA5 loci and hemostatic parameters derangement on the development of atherosclerosis and CCVD.

Authors' contributions: CNF, MGC, MOS, APF, and AP were involved with the conception and organization of the research project. CNF, HJR, and KBG executed the studies. CNF and HJR designed and carried out the statistical analyses and wrote the first draft of the manuscript. MOS reviewed the statistical calculations, and AP and MOS revised the article. The co-authors Palotás and Sousa are equal in status.

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