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Research Article

HPA-3 and *C807T* polymorphisms are associated with laboratory biomarkers of coronary artery disease in Brazilian women

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Abstract

Reports describe the association between platelet activation, adhesion, and aggregation, as well as polymorphisms in genes encoding platelet membrane glycoproteins with a predisposition to coronary artery disease (CAD). This study investigates associations between HPA-1, -3 and C807T polymorphisms and CAD. A cross-sectional study involving 114 women who underwent coronary angiography was performed. The CAD+ group consisted of 63 women while the CAD- group of 51 women. Hematological and biochemical parameters were evaluated by electronic methods. Polymorphisms were investigated by PCR-RFLP and PCR-SSP and participants answered an epidemiological questionnaire and provided anthropometric data. Blood pressure was also measured. Statistical analyses were performed using EpiInfo v. 7.0 software. Results showed that age, diabetes mellitus, hypertension, and hyperlipidemia were associated with CAD. In addition, CAD+ women showed elevated glucose, TG, and VLDL-c levels and reduced HDL-c levels. No significant difference was observed between the allelic and genotypic distributions of the polymorphisms HPA-1, -3, and C807T in CAD+ and CAD- groups. However, CAD+ women carriers of the variant 3b allele had elevated TG and sP-selectin levels while the carriers of the variant 807T allele had reduced CT and LDL-c levels. Hence, our data suggest that the polymorphism HPA-3 increases, indirectly, the risk of CAD development and occurrence while polymorphism C807T reduces this effec.

Introduction

Coronary Artery Disease (CAD) is an atherosclerotic event characterized by chronic vascular inflammation. Risk factors such as metabolic disorders (hypertension, hypercholesterolemia, homocystinuria, obesity, and diabetes mellitus) and environmental factors (smoking and psychosocial stress) are predictors of cardiovascular diseases [1]. In addition, genetic factors have been identified as a predictor of death due to CAD in young individuals because single nucleotide polymorphisms in genes of Human Platelet Antigens (HPA) have been observed in patients with premature myocardial infarction [2,3].

CAD is more prevalent among men than women, however, this is changing as the estimated life expectancy of Brazilians is increasing and especially among females and it is estimated that women live 7.8 years longer than males, surpassing an average age of 75 years [4,5]. In the United States and worldwide, CAD is one of the most important causes of death among women. The onset of menopause increases cardiovascular risk in older women and is also very serious in cases of early menopause; in these cases, CAD can be considered independent of other classic risk factors [6].

Platelet activation and aggregation play a key role in the atherosclerotic process and studies have demonstrated that polymorphisms in genes encoding platelet membrane glycoproteins (GPs) may influence the platelet aggregability

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and increase the risk of arterial thrombosis and atherosclerosis [7,8]. Polymorphisms in genes encoding platelet receptors GPIIIa (*HPA-1;* -1565C > T; rs4918), GPIIb (*HPA-3;* -843T > G; rs5911), and GPIa (-807C > T; rs1126643) have been associated with a thrombotic state, by altering the platelet structure, increasing the expression of receptors as well as the affinity of collagen and other binding factors in individuals with cardiovascular diseases [9,10]. The relationship, however, between polymorphisms in genes encoding platelet receptors and complications of CAD remains controversial, as revised by Strisciuglio and others [11]. Therefore, this study investigated the presence of platelet glycoproteins IIb/IIIa and Ia/IIa polymorphisms and other inflammatory markers in women from Salvador, Bahia, and Brazil.

Material & methods

Study design

The present cross-sectional study included 114 women who underwent Coronary Angiography (CA) at the outpatient clinic in Ana Nery Hospital, a specialized treatment center for Coronary Disease located in Salvador, Bahia-Brazil. CAD was defined as ≥ 70% stenosis in at least one segment of one of the major epicardial arteries or ≥ 50% stenosis in the left coronary artery [12]. According to the Brazilian Stable Coronary Disease Guideline, women were classified into CAD+ and CAD- groups [12]. The CAD+ group consisted of 63 women, aged between 39 and 81, who presented at least one of the criteria for CAD. The CAD- group consisted of 51 women, aged between 40 and 65 years, who did not meet the CAD criteria, and women with no family history of cardiovascular disease. Women with inflammatory, infectious, neoplastic, or congenital cardiac disease were excluded from the study.

Ethical aspects

The present study received approval from the Institutional Review Board of the Nursing School of the Federal University of Bahia (EE-UFBA) and complies with the Declaration of Helsinki 1964 and its revisions. Besides, all women provided a signed term of informed consent.

Demographic and anthropometric data

Demographic data and medical history were collected using a standardized and confidential questionnaire (self-reported) completed by each patient. Weight was measured using a mechanical anthropometric scale (Welmy, São Paulo, Brasil) (100 g accuracy) and height was assessed using a fixed scale (1 cm accuracy) in a standing position. Body mass index (BMI) was calculated by dividing weight (in kilograms) by the square of height (Kg/m2) and obesity was defined as BMI ≥ 30. Waist circumference (WC) was measured using an appropriate tape measure with 0.1 cm accuracy. Blood pressure (BP) readings were obtained from each participant after sitting in a relaxed position for at least 5 min. BP was measured three times at 5 min intervals, and the average of these measurements was used for analysis. Hypertension was defined as BP ≥140/90 mmHg and/or the use of antihypertensive drugs. Diabetes mellitus was considered fasting blood glucose ≥ 126 g/dl or insulin use. At the same time, hyperlipidemia was defined as the use of anti-lipid drugs or total cholesterol ≥ 200 mg/dl and/or triglycerides ≥ 150 mg/dl.

Laboratory analysis

Laboratory and molecular analyses were carried out at the Clinical and Toxicological Analysis Laboratory of the College of Pharmaceutical Sciences, Federal University of Bahia (LACTFAR-UFBA). Hematological analyses were performed using a Cell Dyn-Ruby electronic cell counter (Abbott Diagnostics, Wiesbaden, Germany). Lipid profiles and glucose levels were assessed by an immunochemistry assay using an automated spectrophotometer A25 autoanalyzer (Biosystems SA, Barcelona, Spain). C-reactive protein (C-RP) levels were assessed by an immunoturbidimetric method (Wiener Lab. Group, Buenos Aires, Argentina) and sP-selectin by an enzyme-linked immunosorbent assay (ELISA) (Invitrogen, Life Technologies, California, USA). Fibrinogen levels were quantified using a Destiny Plus analyzer (Trinity Biotech, Texas, USA). All immunoassays were performed in duplicate and plates were read on an ELISA reader (TP-Thermoplate Reader) according to the manufacturer's recommendations.

Molecular analyses

Molecular analyses were carried out on genomic DNA extracted from peripheral blood using a Flexigen 250 kit (Qiagen, Hilden, Germany). Polymorphisms *HPA-1* (-1565C>T) and *HPA-3* (-843T>G) were investigated by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis using the *Hpa*II and *Fok*I restriction enzymes, respectively [13]. Polymorphisms *C807T* (-807C>T) were investigated by a single specific primer-PCR (SSP-PCR) [14].

Statistical analysis

Statistical analyses were performed using EpiInfo 7.0 software, with p-values lower than 0.05 assumed to be statistically significant. The Shapiro-Wilk test was used to determine the quantitative variable distribution and the comparison of the mean values between the two groups was performed using the unpaired t-test (normal distribution) and Mann-Whitney U-test (non-normal distribution). Fisher's Exact test or Chi-square test (2-test) with Yates correction was performed for qualitative variables. Odds ratios with 95% confidence intervals (CI 95%) were calculated. Association analysis between laboratory parameters and polymorphisms, using a genetic dominant model and multivariate linear regression analysis was performed to evaluate the influence of *HPA*-1, *HPA*-3, and *C807T* polymorphisms on laboratory parameters. When appropriate, results were expressed as means ± standard deviation (M ± SD), numerical values, or percentages.

Results and discussion

Clinical and demographic characteristics of the women

This study investigated the presence of HPA-1, -3, and C807T gene polymorphisms and other inflammatory markers

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in women. Initially, clinical and demographic characteristics among CAD+ and CAD- women were compared (Table 1).

Results showed that the median age of CAD+ women was significantly higher than that of the CAD- women. This finding corroborates results reported by other studies performed in individuals with cardiovascular disease [15-17] and confirms the chronicity of CAD and the progressive development of atherosclerotic plaque in older women. Moreover, many studies have shown that menopausal women face an increased risk of developing cardiovascular disease due to reduced levels of estrogen, despite the use of hormone therapy did not reduce cardiovascular risks for cases associated with premature menopause [5,18]. The mechanism of protection induced by this hormone in younger women is not well understood. However, it is known that estrogen binding to endothelial cells activates lipid metabolizing enzymes, accelerates chylomicron removal and the capture of LDL-c and VLDL-c, which facilitates bile acid secretion, the removal of cholesterol, increases Apo-A synthesis and raises plasma HDL-c levels [19]. Furthermore, CAD+ women presented higher frequencies of metabolic syndrome-associated conditions such as diabetes mellitus, hypertension, and hyperlipidemia, as demonstrated in this study. This study observed that among CAD- women, 22 (43.1%) had metabolic syndrome, compared to 44 (69.8%) CAD+ women. These results confirm the significant association between metabolic syndrome and cardiovascular disease (OR = 3.05, 95% CI = 1.41-6.61, p = 0.0073). Metabolic syndrome consists of clinical conditions associated with cardiometabolic risk factors due to insulin resistance, chronic hyperglycemia, and dyslipidemia, which predispose women to endothelial dysfunction, systemic inflammation, and prothrombotic states. Women in menopause have a higher prevalence of metabolic disturbances than men because of hormonal changes. Individuals with metabolic syndrome present more risk of dying from cardiovascular disease than others with no metabolic syndrome [20,21,22,23].

Laboratory parameters of the women

The hematological, biochemical, and inflammatory parameters of the CAD+ and CAD- women are shown in Table 2.

Studies have demonstrated the relationship between white blood cell (WBC) count and CAD. Moreover, neutrophils and lymphocytes are associated with the genesis and evolution of atheroma plaques [24]. In this study, analysis of the hematological parameters showed higher lymphocyte count in CAD+ than CAD- patients, without differences in neutrophil count. These results were not expected since the neutrophils increase in the systemic inflammatory state, while the lymphocytes reduce acute stress [24]. These differences can be explained due to the group of women studied since women classified as CAD- had several cardiovascular risk factors, such as diabetes mellitus, hypertension, and hyperlipidemia. Although elevated levels of cortisol can induce the reduction of lymphocytes by apoptosis during cardiovascular disease evolution, the exact mechanism of this decrease has not been fully clarified [25].

Table 1: Demographic and clinical characteristics of the study population.

	CAD⁺ (n:63)	CAD⁻ (n:51)	*p*-value
Age, Median (minimum-maximum)	61 (39-81)	55 (40-65)	< 0.01*
Diabetes Mellitus, n (%)	33 (52.4%)	14 (27.4%)	0.01**
Hypertension, n (%)	62 (98.4)	43 (84.3%)	0.01***
Hyperlipidemia, n (%)	50 (79.4%)	31 (60.8%)	0.03**
Smoker, n (%)	38 (60.3%)	21 (41.2%)	0.06***
SBP (mmHg), M ± SD	148 ± 29	142 ± 23	0.2*
DBP (mmHg), M ± SD	81 ± 13	80 ± 13	0.6*
BMI (kg/m²), M ± SD	29.3 ± 5.5	29.7 ± 5.5	0.7*
WC (cm), M ± SD	101.3 ± 13	99.5 ± 12	0.5*

SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; BMI: Body Mass Index; WC: Waist Circumference; CAD⁺: women with CAD; CAD⁻: women without CAD; *Unpaired t-test; **X^2- Corrected Yates; *** Fisher's exact test; M: Mean; SD: Standard Deviation; n: number

Table 2: Biochemical, inflammatory, and hematological parameters of the study population.

	CAD⁺ (n:63) (M ± SD)	CAD⁻ (n:51) (M ± SD)	*p - value*
RBC (× 10⁶/cu mm)	4.7 ± 0.4	4.6 ± 0.4	0.5*
Hemoglobin (g/dL)	13.8 ± 0.3	13.6 ± 1.4	0.6**
Hematocrit (%)	41.4 ± 3.0	40.8 ± 4.4	0.7**
MCV (fL)	88.6 ± 5.2	88.8 ± 4.8	0.8*
MCH (pg)	29.5 ± 2.1	29.5 ± 1.7	0.9*
MCHC (%)	33.3 ± 1.0	33.2 ± 0.9	0.7*
RDW (%)	12.7 ± 0.9	12.5 ± 0.7	0.4*
Leukocyte Count (x10³/mL)	7.5 ± 2.2	7.0 ± 1.7	0.2*
Neutrophil Count (x10³/mL)	4.1 ± 1.5	4.1 ± 1.4	0.9*
Lymphocyte Count (x10³/mL)	2.5 ± 0.8	2.2 ± 0.7	0.02*
Eosinophil Count (/mL)	173 ± 99	174 ± 122	0.9*
Basophil Count (/mL)	83 ± 38	85 ± 51	0.5**
Monocyte Count (/mL)	513 ± 177	486 ± 158	0.4*
Platelet Count (/mL)	219.258 ± 5.6198	222.740 ± 5.1173	0.7*
MPV (fL)	8.8 ± 1.6	8.6 ± 2.1	0.5*
PDW (%)	20.6 ± 1.5	20.4 ± 1.3	0.3*
Plateletcrit (%)	0.195 ± 0.051	0.182 ± 0.043	0.2*
Glucose (mg/dL)	133.5 ± 61.7	105.3 ± 23.7	0.03**
Triglycerides (mg/dL)	158.6 ± 73.7	113.8 ± 50.1	< 0.01**
TC (mg/dL)	193.8 ± 55.97	195.2 ± 47.85	0.5**
HDL-c (mg/dL)	42.3 ± 10.0	49.6 ± 13.3	<0.01**
LDL-c (mg/dL)	108 ± 38.1	123.9 ± 38.6	0.1**
VLDL-c (mg/dL)	31.64 ± 14.5	23.6 ± 11.8	< 0.01**
C-Reactive Protein (mg/dL)	3.7 ± 1.9	4.1 ± 2.7	0.6**
Fibrinogen (mg/dL)	374 ± 83	370 ± 82	0.7*
sP-selectin (pg/mL)	8.4 ± 3.6	6.9 ± 4.2	0.04*

RBC: Red Blood Cell; MCH: Mean Corpuscular Hemoglobin; MCV: Mean Corpuscular Volume; MCHC: Mean Corpuscular Hemoglobin Concentration; RDW: Red Cell Distribution Width; MPV: Mean Platelet Volume; PDW: Platelet Distribution Width; TC: Total Cholesterol; HDL-c: High-Density Lipoprotein Cholesterol, LDL-c: Low-Density Lipoprotein Cholesterol, VLDL-c: Very Low-Density Lipoprotein Cholesterol; CAD⁺: women with CAD; CAD⁻: women without CAD; *Student's T-test; **Mann Whitney U-test; M: Mean; SD: Standard Deviation.

Analysis of the biochemical parameters showed elevated glucose levels in CAD+ women compared to CAD- women. These results may be a consequence of the presence of diabetes mellitus in 52.4% of CAD+ women, compared to 27.4% of CAD- women; in addition, among DAC+ women with diabetes mellitus, only 41.2% were on acceptable use of glycemic-lowering therapy.

Besides, CAD+ women presented a significant increase in TG and VLDL-c levels and significantly decreased HDL-c

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compared to CAD- women. However, all laboratory exam results fell within the normal limits established by the Brazilian Guidelines for Dyslipidemia and Prevention of Atherosclerosis [19]. Therefore, these findings may be due to the appropriate use of lipid-lowering drugs, as observed in 71% of women with CAD+.

The analysis of inflammatory markers found higher concentrations of sP-selectin in CAD+ women than CAD- women (p = 0.04). sP-selectin is an adhesion molecule found in platelet granules and Weibel-Palade bodies of human endothelial cells [26,27]. An experimental study carried out in mice with atherosclerosis showed that endothelial and platelet P-selectin contributed to the exacerbation of atherosclerotic lesions via the recruitment of monocytes and other leukocytes, in addition to the formation of platelet-leukocyte complexes. This fact facilitated endothelial cells' expression of inflammatory mediators [28]. In the present study, we observed that CAD+ women had elevated sP-selectin levels compared to CAD- women. This finding showed the participation of activated platelets in cardiac events in individuals with the acute coronary syndrome (ACS) and corroborated previous studies [5,15].

Genotypic and allelic distribution of the polymorphisms HPA-1, HPA-3 and C807T

Tables 3,4 show the genotypic and allelic distributions of polymorphisms *HPA-1*, *HPA-3*, and *C807T* in CAD+ and CAD- groups; the polymorphisms investigated were found to be in Hardy-Weinberg equilibrium (2 - test, $p > 0.05$). Studies involving polymorphisms in *GPs* and relevant associations with CAD remain controversial. The present study found no association between the occurrence of CAD and polymorphisms *HPA-1* or -3, nor the silent polymorphism *C807T*.

Table 3: Genotypic frequencies of polymorphisms *HPA-1, -3,* and *C807T* in CAD$^+$ and CAD$^-$ women.

	***HPA-1* (-1565C>T)**			
GENOTYPE	CAD$^+$ n (%)	CAD$^-$ n (%)	OR (95% CI)	*p* - value
1a1a	46 (75.41)	41 (82)	1.48 (0.59-3.76)	0.54*
1a1b/1b1b	15 (24.59)	9 (18)		
	HPA-3 (-843T>G)			
GENOTYPE	CAD$^+$ n (%)	CAD$^-$ n (%)	OR (95% CI)	*p* - value
3a3a	29 (50)	23 (50)	1.00 (0.46-2.16)	1.0*
3a3b/3b3b	29 (50)	23 (50)		
	C807T (-807C>T)			
GENOTYPE	CAD$^+$ n (%)	CAD$^-$ n (%)	OR (95% CI)	*p* - value
CC	30 (48,4)	26 (52)	1.1 (0.54-2.43)	0.8*
CT/TT	32 (51,6)	24 (48)		

CAD$^+$: women with CAD; CAD$^-$: women without CAD; *X^2: Yates correction; OR: odds ratio; 95% CI: 95% confidence intervals.

Table 4: Allelic frequencies of polymorphisms *HPA-1, -3,* and *C807T* in CAD$^+$ and CAD$^-$ women.

***HPA-1* (-1565C>T)**				
ALLELE	CAD$^+$ n (%)	CAD$^-$ n (%)	OR (95% CI)	*p* - value
1a	107 (87.7)	91 (91)	1.4 (0.60-3.40)	0.6*
1b	15 (12.3)	9 (9)		
HPA-3 (-843T>G)				
ALLELE	CAD$^+$ n (%)	CAD$^-$ n (%)	OR (95% CI)	*p* - value
3a	78 (67,2)	63 (68.5)	1.0 (0.58-1.90)	0.9*
3b	38 (332,8	29 (31.5)		
C807T (-807C>T)				
ALLELE	CAD$^+$ n (%)	CAD$^-$ n (%)	OR (95% CI)	*p* - value
C	80 (64,5)	71 (71)	1,3 (0.76-2.37)	0.3*
T	44 (53,5)	29 (29)		

CAD$^+$: women with CAD; CAD$^-$: women without CAD; *X^2: Yates correction; OR: odds ratio; 95% CI: 95% confidence intervals.

Thrombotic events and the development of acute coronary syndrome are associated with platelet activation, aggregation, and adhesion, dependent on platelet membrane glycoprotein receptors [29]. Some studies have demonstrated an association between an increased thrombotic state and polymorphisms in *GPs* platelets since it seems to alter platelet structure and increase the expression of receptors with a high affinity to collagen [9,30]. Despite this, studies involving HPA polymorphisms are still controversial. For example, a study in Iran showed no association of alleles, genotypes, and haplotypes of HPA with cardiovascular risk, but HPA1b/2a/3b haplotype was considered a dependent risk after correction for confounding factors [31]. Additionally, some factors, such as criteria used to define CAD in patients and studies with different ethnic populations, may be contributing to conflicting results involving studies on HPA-1b/2a/3b alleles, genotypes, haplotypes, and association with CAD.

Association analysis between the investigated polymorphisms and laboratory parameters

Although the polymorphisms investigated in the present study did not demonstrate a direct association with CAD, they seem to be associated with biomarkers directly involved in cardiovascular disease occurrence. Table 5 lists the results of the associations between the *HPA-1*, *HPA-3*, and *C807T* polymorphisms and biochemical and inflammatory parameters in CAD+ women. *HPA*-1 polymorphism did not demonstrate any significant association with laboratory parameters in CAD+ women, suggesting that this polymorphism may not be associated with coronary atherosclerosis in the studied population. Together with the evidence that CAD+ women had higher levels of TG in comparison with CAD- women, association analysis showed that CAD+ women carriers of the variant 3b allele of the polymorphism *HPA-3* had significantly elevated TG levels compared to those with the wild-type 3a allele. A report demonstrated that elevated blood lipids, particularly LDL-c and TG, contribute to the formation of atherosclerotic plaques and an inflammatory process that can lead to stroke [32]. A study with the Iranian population showed a positive association of the HPA1b/2a/3b haplotype with CAD after adjustment for some covariates, such as BMI, TG and LDLc [30]. Furthermore, CAD+ women with the variant 3b allele had significantly elevated sP-selectin levels compared to those with the wild-type 3a allele. sP-selectin is a biomarker of platelet activation and is considered a risk factor for atherosclerotic disease as it contributes to the formation of atherosclerotic plaque due to increased platelet activation and aggregation [26].

Contrary to *HPA*-3, *C807T* was significantly associated with reduced CT and LDL-c levels in CAD+ women. Then, taking into account the crucial role of lipids in the atherosclerotic process, primarily increased CT, LDL-c, VLDL-c, or TG levels,

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Table 5: Laboratory parameters in CAD+ women according to the genotypic distribution of three polymorphisms.

Laboratory Parameters	*HPA-1* (-1565C>T) M ± SD			*HPA-3* (-843T>G) M ± SD			*C807T* (-807C>T) M ± SD		
	1a1a (n:46)	1a1b/1b1b (n:15)	*p* value	3a3a (n:29)	3a3b/3b3b (n:29)	*p* value	CC (n:30)	CT/TT (n:32)	*p* - value
TC (mg/dL)	190.8 ± 56.3	202.3 ± 57.1	0.500*	186.9 ± 51.2	200.2 ± 60.5	0.388*	212.2 ± 59.1	176.9 ± 50.8	0.019*
LDL-c (mg/dL)	115.2 ± 52.2	125.8 ± 51.8	0.513*	117.6 ± 50.4	119.1 ± 54.3	0.917*	133.5 ± 51.6	104.8 ± 49.6	0.042*
HDL-c (mg/dL)	41 ± 9.2	45.1 ± 11.4	0.172*	42.4 ± 11.3	41.4 ± 8.2	0.696*	45.0 ± 10.6	40.0 ± 8.7	0.063*
TG (mg/dL)	170.0 ± 88.9	176.7 ± 145.0	0.834*	142.5 ± 66.8	199.1 ± 124.2	0.048**	168.0 ± 115.2	165.3 ± 95.9	0.924*
Glucose (mg/dL)	134.9 ± 69.0	151.3 ± 81.0	0.462*	129.8 ± 69.1	148.0 ± 75.4	0.351*	131.4 ± 71.8	141.1 ± 74.1	0.627*
sP-selectin (pg/mL)	8.5 ± 3.9	9.0 ± 3.9	0.650*	7.5 ± 3.6	9.6 ± 4.3	0.041*	8.0 ± 3.2	9.2 ± 4.5	0.266*

TC: Total Cholesterol; HDL-c: High-Density Lipoprotein Cholesterol, LDL-c: Low-Density Lipoprotein Cholesterol; TG: Triglycerides; *unpaired t-test; **Mann Whitney U-test; M: Mean; SD: Standard Deviation; n: number.

Table 6: Multivariate linear regression models of genetic polymorphisms and confounding variables on laboratory parameters in CAD+ women.

Independent Variables	Dependent variable	B	*p* - value	R	*a p* - value of the model
Model					
*HPA-3**	sP-Selectin	2,623	0,013	0,423	0,014[a]
Hypertension		8,991	0,024		
Diabetes Mellitus		0,872	0,368		
Model					
*C807T**	TC	-36,483	0,014	0,378	0,036[a]
Hypertension		-44,638	0,437		
Medication[#]		-17,365	0,364		
Model					
*C807T**	LDL-c	-32,673	0,016	0,328	0,044[a]
Hypertension		-50,366	0,324		

TC: Total Cholesterol; LDL-c: Low-Density Lipoprotein Cholesterol; R: Coefficient of determination; β: coefficient of regression; [a]ANOVA; [‖] Dominant genetic model, [#] use lipid-lowering drugs

this finding suggests the association of the variant 807T allele with the reduction in the risk of developing coronary atherosclerosis. However, a preview study carried out in a Chinese population with ischemic stroke found an association between the variant 807T allele and elevated LDL-c and TG levels [32]. Furthermore, a previous study showed that the C807T polymorphism influences the expression of glycoprotein GPIa in the platelet membrane and the development of CAD, varying significantly between individuals and different populations [33]. This discrepancy may be due to factors inherent to the participants, such as ethnicity and clinical plurality, in addition to each study's sample size.

Based on these findings, we performed a multivariate analysis in the CAD+ group, adjusted according to confounding variables (Table 6). The association of the HPA3b allele with elevated TG levels in CAD+ women was not validated after multivariate regression analysis, adjusted for hypertension and lipid-lowering drugs. However, the association of the HPA3b allele with elevated serum levels of sP-selectin remained significant when it was adjusted for the risk factors associated with CAD development. Logistic regression analysis confirmed the association of the 807 T allele with reduced serum CT and LDL levels; however, the data on LDL levels were not significant when they were adjusted for lipid-lowering drugs.

Conclusion

The present study confirms age, glucose, and lipid profile association with CAD. Despite the small sample size of this study, the variant 3b and 807T alleles of *HPA-3* and *C807T* polymorphisms, respectively, were associated with biomarkers directly related to the formation of atherosclerotic plaques and inflammatory process and, consequently, cardiovascular events occurrence.

Furthermore, these polymorphisms seem to have opposite effects in the women investigated: *HPA-3* increases, indirectly, the risk of CAD development and occurrence while *C807T* reduces this effect. However, further studies of clinical follow-up among these women, as well as studies with men, are needed to establish a cause-effect relationship between *HPA3* and *C807T* polymorphisms and CAD development.

Data availability

All relevant data are within the manuscript.

Author contributions

Suellen Pinheiro Carvalho Valverde, Elisângela Vitória Adôrno, and Marilda de Souza Gonçalves conceived and designed the study and performed statistical analyses. Suellen Pinheiro Carvalho Valverde and Karina Oliveira Mendes collected the samples and performed all experiments. Setondji Cocou Modeste Alexandre Yahouédéhou, Rodrigo Oliveira Mota, Cleverson Alves Fonseca, and Nathalie Souza Veloso assisted in some experimentation and provided technical support. Suellen Pinheiro Carvalho Valverde, Elisângela Vitória Adôrno, Marilda de Souza Gonçalves, and Cynara Gomes Barbosa discussed the results, wrote the manuscript, and critically revised the manuscript. All authors revised and approved the final version of the manuscript. acquired the funding.

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