Smoking-dependent and haplotype-specific effects of endothelial nitric oxide synthase gene polymorphisms on angiographically assessed coronary artery disease in Caucasian- and African-Brazilians

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Abstract

The nitric oxide produced by endothelial nitric oxide synthase (eNOS) plays a pivotal role in protecting the arterial wall from damages and atherosclerosis. The T–786C, the 27-bp repeat in intron 4, and the E298D eNOS gene polymorphisms were studied in 715 Brazilian patients (447 Caucasian- and 268 African-Brazilians) who underwent coronary angiography. The −786C frequency was increased in coronary artery disease (CAD) cases with significant lesions ($\geq$50% luminal obstruction) when compared with lesion-free controls; this difference was detected in smokers but not in nonsmokers, both in Caucasian- ($p = 0.011$) and African-Brazilians ($p = 0.005$). The interaction between −786C carriers and smoking was an independent CAD predictor (OR: 2.9, 95% CI: 1.4–5.9; $p = 0.003$) in multiple logistic regression. The 298D mutation frequency was also higher among CAD cases ($p = 0.036$) in African-Brazilian smokers, but this effect was not independent from other variables in the regression model. Though not associated with CAD, the 4-repeat allele combined with different T–786C alleles showed protective and susceptible effects in Caucasian-Brazilian smokers. The −786C/4-repeat/298E haplotype frequency was higher ($p = 0.020$), whereas −786T/4-repeat/298E was lower ($p = 0.023$) in these cases. These results showed a smoking-dependent effect of the T–786C eNOS polymorphism on CAD in both Caucasian- and African-Brazilians. Additionally, the haplotype analysis revealed different eNOS haplotypes associated with protection and susceptibility to the disease.
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1. Introduction

Coronary artery disease (CAD) is the leading cause of death in the world. The underlying pathogenesis involves several environmental and genetic factors that also interact among themselves causing the development of atherosclerotic plaque [1]. Nitric oxide, synthesized by endothelial cells and platelets from ʟ-arginine, has a key role in the endothelial function protecting the arterial wall from developing atherosclerotic lesions. This metabolite acts in lowering the arterial tonus, inhibits platelet aggregation and thrombus formation. It also limits the arterial wall-inflammatory response to risk factor damages by inhibiting the leukocyte adhesion and migration through the endothelium and lowering the lipoprotein particle oxidation [2].

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The endothelial nitric oxide synthase (eNOS) gene presents some polymorphisms that have been previously associated with angiographically assessed CAD or myocardial infarct (MI) [3–5]. A functional variant in the eNOS promoter (T–786C) is associated with reduction in the promoter efficiency and the level of expressed enzyme leading to increased CAD risk [6–8]. The eNOS gene also presents a variable number of tandem repeats (27 bp) in intron 4, and the rare 4-repeat allele was associated with CAD [9]. Another common eNOS mutation is located in exon 7 and causes the change of glutamine (E) to aspartic acid (D) in codon 298. The 298D allele has been previously associated with endothelial function [10] and CAD risk in some [11–13] but not all studies [14]. Additionally, few studies have addressed the combined effect of these three eNOS polymorphisms forming haplotypes and their effects on cardiovascular disease, and some of these investigations showed protective and susceptible eNOS haplotype effects [8,15].

As predicted by the multifactorial nature of CAD, some candidate gene effects on disease phenotypes seem to be triggered by environmental factors [1,16]. Smoking is a modifiable CAD risk factor that accelerates atherosclerotic plaque development and premature CAD by endothelial damage leading to reduced endothelial function [17]. Cigarette smoke directly lowers eNOS protein level and modifies enzyme activity in vivo [18]. These effects seem to differ depending on the eNOS variant because smoking-dependent eNOS genotype associations with CAD have been reported [9,10,18].

Ethnic background is known to influence polymorphism frequencies and their effects on the disease. The great majority of eNOS studies were done on European or North American Caucasians and few studies have addressed the eNOS effects in Africans or their descendents [3,4]. The aim of the present study was to investigate the eNOS polymorphisms and haplotypes, and the effects of their interaction with smoking over angiographically assessed CAD in African- and Caucasian-Brazilians.

2. Methods

2.1. Subjects

A total of 715 patients (447 Caucasian-Brazilians and 268 African-Brazilians) who underwent coronary angiography due to symptoms related to CAD were studied. They were contacted at the homodynamic Unit of Santa Izabel's Hospital in Salvador, Bahia State, Brazil. Cardiologists not aware of patient genotypes interpreted the angiograms. Two hundred and sixty seven patients presented obstructive lesion-free examinations and were included as controls in our analysis. Four hundred and forty eight patients presenting ≥50% obstructive lesions were taken as CAD cases; among them 200 patients also presented previous MI history confirmed by ECG and/or cardiac enzymes in their medical records.

Patients with intermediate disease (obstructive lesions <50) were excluded from the study.

The population from Bahia State, in northeastern Brazil, is composed of a significant number of African descents because this state had been the major destination of African slaves in Brazil during the colonial period; the Caucasians are mostly of Portuguese descent [19]. The African ancestry was assessed by morphological facial characteristics and skin pigmentation in the interior part of the forearm as previously reported and validated to our population by classical genetic markers [20]. Using these criteria, patients were classified into Caucasian-Brazilians and African-Brazilians.

The patients underwent physical examination and provided a detailed medical history. Individuals were defined as hypertensive if their blood pressure was >140/90 mm Hg or if they were receiving any antihypertensive treatment. Individuals with a fasting glucose ≥126 mg/dl or those receiving any antidiabetic medication were considered diabetic. Family history was considered positive for CAD if at least one first-degree male relative was diagnosed with angina, MI, or sudden death by the age of 55 or one first-degree female relative was diagnosed with CAD by the age of 65 years. Smoking was defined as self-reported, current or past smoking. All subjects provided informed written consent approved by the Hospital Ethics Committee.

2.2. Genotype and biochemical analysis

Blood samples were collected for genomic DNA extraction by salting-out procedure. The T–786C and the E298D eNOS genotypes were detected by polymerase chain reactions (PCRs) followed by restriction fragment length analysis as previously described [7,11]. Homozygotes to the restriction site were used as positive controls in all reaction digestion. The 27-bp repeat polymorphism in intron 4 was detected as described by Alvarez et al. [7]. In all procedures, DNA fragments were separated by 8% polyacrylamide gel electrophoresis and visualized under ultraviolet light after ethidium bromide staining.

Total cholesterol, high-density-lipoprotein cholesterol (HDL-c), triglycerides, and glucose serum levels were measured by enzymatic methods in an autoanalyzer device using commercial kits (Weiner) in subjects under at least 12-h fasting. The low-density-lipoprotein cholesterol (LDL-c) levels were calculated by Friedewald formulae [21].

2.3. Statistical analysis

Allele frequencies were estimated by gene counting. The agreement of genotype frequencies with Hardy–Weinberg expectations and the pairwise linkage disequilibrium were tested by a χ^2 goodness of fit test using Arlequin program, Version 2.000 [22]. This program was also used to estimate haplotype frequencies in CAD cases and controls from genotype frequencies using maximum likelihood methods, the estimations were done separately for Caucasian- and African-

Brazilians. The D' values, the relative magnitude of disequilibrium (D) when compared with its theoretical maximum value (D/D_{max}), were calculated as described by Lewontin [23]. The Mann–Whitney U-test was used to compare quantitative variables between groups. Allele and haplotype frequency differences between cases and controls were tested by Pearson χ^2 test using the PEPI program, Version 4.0 [24]. The haplotype analyses were followed by an adjusted χ^2 residual test to establish haplotypes that were responsible for the difference. The odds ratio estimations and the multiple logistic regressions were performed using the SPSS program Version 10. A p-value <0.05 was considered statistically significant.

3. Results

Clinical and demographic characteristics of CAD cases and controls were summarized in Table 1. Cases and controls presented similar total and LDL-c levels, but triglycerides were higher and HDL-c levels were lower in cases when compared with those of controls. As expected, cases also presented higher diabetes mellitus, smoking, and early CAD family history prevalence, and were somewhat older than controls. Cases and controls were similar in the number of studied years, body mass index (BMI), and hypertension prevalence. Differences between cases and controls with respect to ethnic groups were also tested. The results showed that African-Brazilians were underrepresented among CAD cases (32.8%) when compared with controls (45.3%; $p < 0.001$).

All the eNOS genotype frequencies were according to those expected by the Hardy–Weinberg equilibrium in both ethnic groups. In African-Brazilian controls, the −786C, the 298D, and the 4-repeat alleles frequencies were 21.6%, 22%, and 19.8%, respectively, whereas among Caucasian-Brazilian controls these frequencies were 32.9%, 26%, and 22.6%, respectively. These frequency differences

Table 1
Clinical and demographic characteristics of CAD cases and controls

Variable	CAD cases[a]	Controls[a]	p
Number	448	267	–
Age	55.6 ± 7.1	52.5 ± 8.2	<0.001
Gender (female/male)	148/300	143/124	<0.001
Diabetes mellitus	131/317	27/240	<0.001
Hypertension	335/113	182/85	0.068
Smoking[b]	255/193	121/146	0.003
Package/year	17.6 ± 23.6	12.8 ± 21.4	0.001
Early CAD family history	169/279	69/198	0.001
Total cholesterol (mg/dl)	188.2 ± 51.1	186.1 ± 43.3	0.956
LDL-cholesterol (mg/dl)	125.0 ± 44.6	125.8 ± 39.5	0.546
HDL-cholesterol (mg/dl)	27.8 ± 7.8	30.7 ± 8.6	<0.001
Triglycerides (mg/dl)	176.4 ± 120.7	148.1 ± 85.7	<0.001
Caucasian-/African-Brazilians	147/301	121/146	<0.001
Studied years	8.4 ± 4.9	7.7 ± 4.9	0.092
BMI[c]	26.9 ± 4.24	26.6 ± 4.85	0.239
Angina	333/115	209/58	0.271

[a] For categorical variables the values express the number of affected/non-affected individuals.
[b] Current and past smoking.
[c] Body mass index (BMI): weight/(height)^2.

between ethnic groups were significant for the −786C allele only ($p = 0.001$). The −786C and 298D alleles were linked in both African- ($D' = 0.435$; $p < 0.0001$) and Caucasian-Brazilians ($D' = 0.444$; $p < 0.0001$). Additionally, the 298D and the 5-repeat alleles were linked either among African- ($D' = -1.000$; $p < 0.0001$) and Caucasian-Brazilians ($D' = -0.787$; $p < 0.001$), whereas the −786C and the 4-repeat alleles were linked in Caucasian- ($D' = 0.278$; $p < 0.01$) but not in African-Brazilians.

To avoid possible population stratification, we conducted the following analyses stratified by ethnic groups. As smoking, a known eNOS gene confounding environmental factor, also differed between CAD cases and controls, we further stratified the analyses by this factor.

Tables 2–4 list the single eNOS polymorphism frequencies in CAD cases and controls according to the smoking status in Caucasian- and African-Brazilians. Independent

Table 2
The T–786C eNOS polymorphisms frequencies in Caucasian- and African-Brazilians CAD cases and controls according to smoking status

T–786C genotypes	Smoking				Nonsmoking			
	CAD cases	Controls	χ^2	p	CAD cases	Controls	χ^2	p
Caucasian-Brazilians								
TT	71 (42%)	39 (63.9%)			53 (40.2%)	34 (40%)		
TC	74 (43.8%)	16 (26.2%)			66 (50%)	34 (40%)		
CC	24 (14.2%)	6 (9.8%)			13 (9.8%)	17 (20%)		
C allele	0.3609	0.2295	6.462	0.011	0.3485	0.4000	0.968	0.325
Odds ratio (95% CI)[a]	2.447 (1.336–4.482)			0.004	0.994 (0.570–1.733)			0.982
African-Brazilians								
TT	40 (46.5%)	44 (73.3%)			29 (47.5%)	32 (52.5%)		
TC	39 (45.3%)	14 (23.3%)			22 (36.1%)	23 (37.7%)		
CC	7 (8.1%)	2 (3.3%)			10 (16.4%)	6 (9.8%)		
C allele	0.3081	0.1500	8.765	0.003	0.3443	0.2869	0.683	0.409
Odds ratio (95% CI)[a]	3.162 (1.552–6.445)			0.002	1.218 (0.598–2.478)			0.587

[a] Odds ratio and 95% confidence interval for rarer allele carriers (homozygous + heterozygous) vs. common allele homozygous.

Table 3

The 27-bp repeat eNOS frequencies in Caucasian- and African-Brazilians CAD cases and controls according to smoking status

Intron 4 repeat genotypes	Smoking				Nonsmoking			
	CAD cases	Controls	χ^2	p	CAD cases	Controls	χ^2	p
Caucasian-Brazilians								
5-Repeat 5-repeat	113 (66.9%)	41 (67.2%)			89 (67.4%)	49 (57.6%)		
5-Repeat 4-repeat	51 (30.2%)	19 (31.1%)			36 (27.3%)	27 (31.8%)		
4-Repeat 4-repeat	5 (3%)	1 (1.6%)			7 (5.3%)	9 (10.6%)		
4-Repeat allele	0.1805	0.1721	0.005	0.945	0.1894	0.2647	3.004	0.083
Odds ratio (95% CI)[a]	1.016 (0.545–1.895)			0.960	0.658 (0.374–1.155)			0.145
African-Brazilians								
5-Repeat 5-repeat	53 (61.6%)	32 (53.3%)			38 (62.3%)	43 (70.5%)		
5-Repeat 4-repeat	28 (32.6%)	27 (45%)			22 (36.1%)	17 (27.9%)		
4-Repeat 4-repeat	5 (5.8%)	1 (1.7%)			1 (1.6%)	1 (1.6%)		
4-Repeat allele	0.2209	0.2417	0.075	0.785	0.1967	0.1557	0.452	0.502
Odds ratio (95% CI)[a]	0.712 (0.365–1.388)			0.318	1.446 (0.679–3.078)			0.339

[a] Odds ratio and 95% confidence interval for rarer allele carriers (homozygous + heterozygous) vs. common allele homozygous.

Table 4

The E298D eNOS frequencies in Caucasian- and African-Brazilians CAD cases and controls according to smoking status

E298D genotypes	Smoking				Nonsmoking			
,	CAD cases	Controls	χ^2	p	CAD cases	Controls	χ^2	p
Caucasian-Brazilians								
EE	90 (53.3%)	34 (55.7%)			65 (49.2%)	49 (57.6%)		
ED	66 (39.1%)	22 (36.1%)			53 (40.2%)	28 (32.9%)		
DD	13 (7.7%)	5 (8.2%)			14 (10.6%)	8 (9.4%)		
D allele	0.2722	0.2623	0.008	0.927	0.3068	0.2588	0.939	0.332
Odds ratio (95% CI)[a]	1.105 (0.614–1.991)			0.739	1.403 (0.810–2.429)			0.227
African-Brazilians								
EE	44 (51.2%)	42 (70%)			35 (57.4%)	29 (47.5%)		
ED	36 (41.9%)	16 (26.7%)			19 (31.1%)	30 (49.2%)		
DD	6 (7%)	2 (3.3%)			7 (11.5%)	2 (3.3%)		
D allele	0.2791	0.1667	4.390	0.036	0.2705	0.2787	0.000	1.000
Odds ratio (95% CI)[a]	2.227 (1.111–4.465)			0.024	0.673 (0.330–1.375)			0.277

[a] Odds ratio and 95% confidence interval for rarer allele carriers (homozygous + heterozygous) vs. common allele homozygous.

of the ethnic background, when smoking was referred, the −786C allele frequency was increased in cases (36% in Caucasian-Brazilians and 30.8% in African-Brazilians) when compared with that in controls (23%, $p = 0.011$ in Caucasian-Brazilians and 15%, $p = 0.003$ in African-Brazilians). However, when smoking was negative, the −786C frequency did not differ between cases and controls in both ethnic groups (Table 2). The 298D allele frequency was also increased in CAD cases (27.9%) when compared with that in controls (16.7%; $p = 0.036$) in African-Brazilians but not in

Table 5

The estimated eNOS haplotype frequencies in African- and Caucasian-Brazilians CAD cases and controls according to the smoking status

Haplotype[a]	African-Brazilians				Caucasian-Brazilians			
	Smoking		Nonsmoking		Smoking		Nonsmoking	
	CAD cases	Controls	CAD cases	Controls	CAD cases	Controls	CAD cases	Controls
−786T/5-repeats/298E (%)	46.15[*]	58.97[*]	47.93	48.36	51.34	53.72	49.43	42.01
−786T/4-repeats/298E (%)	13.61	19.66	11.57	12.30	5.97[**]	12.40[**]	7.98	9.47
−786T/5-repeats/298D (%)	10.65	5.13	5.79	11.48	6.27	10.74	7.60	8.28
−786C/4-repeats/298E (%)	7.10	3.42	7.44	3.28	11.34[***]	4.13[***]	9.89	16.57
−786C/5-repeats/298E (%)	6.51	3.42	6.61	8.20	4.78	4.13	4.56	6.51
−786C/5-repeats/298D (%)	15.98	9.40	20.66	16.39	20.30	14.88	20.53	17.16
p		0.043		0.407		0.018		0.256

[a] The rare (less than 1.5%) −786C/4-repeats/298D and −786T/4-repeats/298D haplotypes were excluded from analysis due low number.

[*] Significant adjusted χ^2 residues ($p = 0.033$).

[**] Significant adjusted χ^2 residues ($p = 0.023$).

[***] Significant adjusted χ^2 residues ($p = 0.020$).

Table 6
The multiple logistic regression for CAD risk in Brazilians

Variable	β	S.E.	Wald	Odds ratio	95% CI	p
Age (years)	0.048	0.012	16.094	1.049	1.025–1.074	<0.001
Male gender	1.022	0.197	27.043	2.780	1.891–4.087	<0.001
Early CAD history	0.529	0.196	7.300	1.697	1.156–2.491	0.007
African-Brazilians	−0.523	0.185	8.019	0.592	0.412–0.851	0.005
Diabetes mellitus	1.416	0.255	30.845	4.122	2.500–6.794	<0.001
LDL-c level (mg/dl)	0.005	0.002	5.199	1.005	1.001–1.010	0.023
HDL-c level (mg/dl)	−0.030	0.012	6.227	0.970	0.948–0.994	0.013
Triglycerides level (mg/dl)	0.002	0.001	6.462	1.002	1.001–1.004	0.011
Hypertension	0.497	0.209	5.638	1.643	1.091–2.476	0.018
Smoking	−0.175	0.251	0.485	0.839	0.513–1.374	0.486
−786C eNOS carriers[a]	−0.109	0.249	0.192	0.897	0.551–1.460	0.661
Interaction with smoking[b]	1.077	0.360	8.949	2.935	1.450–5.944	0.003

Variables that do not remain as significant coronary artery disease (CAD) predictors: 298D carriers, interaction between 298D allele carriers (DD + ED) vs. smoking; 4-repeat allele carriers (4-repeat 4-repeat + 5-repeat 4-repeat), interaction between 4-repeat allele carriers vs. smoking; the number of studied years; body mass index (BMI).

[a] CC + TC.

[b] −786C carriers × smoking.

Caucasian-Brazilians, again in a smoking-dependent way (Table 3). The 27-pb repeat (intron 4) eNOS polymorphism was not associated with CAD (Table 4).

Table 5 lists the maximum likelihood estimation of eNOS haplotype frequencies in cases and controls according to the ethnic group and smoking status. Either in African- or Caucasian-Brazilians, the haplotype frequencies differed between cases and controls in a smoking-dependent way. In African-Brazilian smokers, the adjusted χ^2 residues indicated that the −786T/5-repeat/298E haplotype frequency was decreased in cases (46.15%) when compared with that in controls (58.97%; $p = 0.033$). In Caucasian-Brazilian smokers, the −786C/4-repeat/298E haplotype frequency was higher and −786T/4-repeat/298E was lower in cases (11.34% and 5.97%, respectively) when compared with that in controls (4.13%, $p = 0.020$ and 12.40%, $p = 0.023$, respectively).

A multiple logistic regression analysis that included all samples showed evidence that interaction between the −786C carrier genotypes (−786CC + −786TC) and smoking increased the risk of CAD (odds ratio: 2.9, 95% CI: 1.4–5.9; $p = 0.003$) in Brazilians. The interaction was independent and corrected to other risk factors that remained significant in the model, including ethnic groups. The other eNOS polymorphisms and their interaction with smoking were not significantly independent of CAD predictors and could not remain in the model (Table 6).

4. Discussion

This study focused on the important topic of genetic susceptibility to CAD and the environmental factors that modify such associations. The main finding of the present report was to demonstrate the smoking-dependent effect of eNOS gene on angiographically assessed CAD in African-Brazilians for the first time. This study also corroborated a previous report on the eNOS gene association with CAD in Caucasians from the southern-Brazilian region [8], strongly suggesting that the eNOS gene variability influences CAD susceptibility in Brazilians in a smoking-dependent manner regardless of the ethnic background.

Overall, the eNOS allele frequencies showed in controls are in the same range as those previously reported in the same ethnic group among Brazilians [8,15,25] and other populations [7,26]. However, some eNOS polymorphism frequencies were somewhat different from those reported by Marroni et al. [25]. Differences in ethnic background and demographic histories between the Brazilian regions from where these studies were carried out can contribute to the prevailing inconsistencies. In the present report, African- and Caucasian-Brazilians showed similar 298D and 4-repeat eNOS allele frequencies, but the −786C allele was more common in Caucasian-Brazilians as reported previously [15]. The pattern of linkage disequilibrium in the eNOS gene was also similar in both Brazilian ethnic groups, except for the promoter and 27-bp repeat variants that were linked in Caucasians and randomly associated in African-Brazilians as shown by others [15].

The −786C allele was associated with CAD in both Caucasian- and African-Brazilians in a smoking-dependent manner. Alvarez et al. [7] reported similar findings in Caucasian smokers from Spain, and the association in Caucasian-Brazilians also corroborated a previous report in Brazilians of European descent and other populations [6,8]. In Africans and their descendants, there were few studies on eNOS variants and their effect on CAD [26] and there was no analysis yet on the −786C allele interaction with smoking. The 298D allele was not associated with CAD in Caucasian-Brazilians as previously reported [8], but in African-Brazilians, the risk of CAD increased in a smoking-dependent manner. The E298D eNOS variant is a conservative mutation in a region not directly involved with enzyme catalytic function or substrate interaction. As the 298D was linked with the −786C promoter mutation in African-Brazilians, and as it

did not remain as an independent CAD predictor after including the −786C in the multiple logistic regression model, it is possible that its effect on CAD could be due to the linkage with the promoter mutation, as previously reported [8].

Wang et al. [9] were the first to report the 4-repeat allele effect on CAD in a smoking-dependent manner in Australian Caucasians, but single-locus analysis in Caucasian- and African-Brazilians could not reveal such an association. However, haplotype analysis showed that the 4-repeat allele in association with the −786C promoter mutation forming the −786C/4-repeat/298E haplotype increased the CAD risk in Caucasian-Brazilian smokers. Conversely, the 4-repeat allele in association with the −786T variant in −786T/4-repeat/298E haplotype had a protective effect in the same group. This indicates that the effect of 4-repeat variant is modified from risk to protection depending on the haplotype formed with the promoter polymorphism (T−786C). The lack of association of these specific haplotypes and CAD in African-Brazilians may be explained by the fact that the −786C and 4-repeat alleles were not linked in this ethnic group; however, the wild −786T/5-repeat/298E haplotype showed a protective effect on the disease in this group.

Functional assays with the −786C eNOS promoter showed a 50% reduction in transcription efficiency when compared with the −786T promoter. Adding cigarette smoke to the culture media further increased this difference [6,27]. Cigarette smoke is a rich source of exogenous nitric oxide that can affect endogenous nitric oxide by downregulating its production [28]. These findings suggest a functional mechanism to the observed interaction between the promoter T−786C polymorphism and smoking in the effect on CAD risk detected herein. Additionally, tests using luciferase reporter assay constructs with different T−786C promoters and 27-bp repeat variants in intron 4 showed different haplotype effects on transcriptional efficiency modifiable by cigarette smoke, suggesting that the intron 4 repeat has different *cis*-regulating effects on promoter efficiency depending on the T−786C polymorphism and smoking [27].

The results of our study should be interpreted in the context of some limitations. The control group used in the current study comprises lesion-free people referred for the coronary angiogram due to certain coronary disease symptoms such as angina. In the present study, the eNOS polymorphisms were not associated with angina in both lesion-free controls and CAD cases even after stratifying to the smoking status (data not shown) not suggesting this as a potential bias. Although there was a higher male/female ratio in CAD cases than in controls, such differences apparently did not affect the eNOS association detected herein as gender was included in the multivariate logistic regression analysis without modifying the eNOS effect and its interaction with smoking. The stratification of analysis by smoking and ethnic groups did not seem to compromise the statistical power to detect or exclude eNOS association within each stratum. The calculated statistical power under the significance level of 0.05 and using the

number of individuals and ORs detected herein varied from 82.2% to 91.7% among subgroups.

The present study on angiographically assessed CAD in Caucasian- and African-Brazilians suggested that smoking interacted with the −786C eNOS promoter variant increasing the CAD risk in both ethnic groups. For the first time, this effect was demonstrated in an African-derived group. Additionally, in Caucasian-Brazilian smokers, the −786T/4-repeat/298E and the −786C/4-repeat/298E haplotypes presented protective and susceptible CAD effects, respectively, whereas the −786T/5-repeat/298E haplotype had a protective effect among African-Brazilian smokers.

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