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Short Communication

Paraoxonase 1 gene polymorphisms in angiographically assessed coronary artery disease: evidence for gender interaction among Brazilians

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Abstract

Background: Paraoxonases (PON) are members of an enzyme family involved in preventing low-density lipoprotein oxidation and therefore protecting against atherosclerotic plaque formation.
Methods: We studied the Met55Leu and Gln192Arg *PON1* polymorphisms in 712 patients (437 Caucasian- and 275 African-Brazilians) who underwent coronary angiography.
Results: Among Caucasian-Brazilians, the homozygous *55LeuLeu* frequency was higher among patients with significant coronary artery disease (CAD, obstructive lesions $\geq$50%) than among lesion-free controls (51% vs. 30.3%; $p=0.022$) in females, but not in males. The Gln192Arg *PON1* polymorphism was not associated with CAD, although *192GlnGln* homozygotes presented lower high-density lipoprotein (HDL)-cholesterol ($p=0.035$) and higher triglyceride ($p=0.012$) levels than *192Arg* allele carriers among Caucasian-Brazilian males, but not females. No other lipid-genotype association was detected. Multivariate logistic regression corrected for classic CAD risk factors shows that *55LeuLeu PON1* homozygotes were at increased CAD risk (odds ratio OR = 2.852; $p=0.003$) and that this genotype interacted with gender in its association with CAD risk (OR = 0.290; $p=0.006$) among Caucasian-Brazilians.
Conclusions: This report shows that the *55LeuLeu PON1* genotype increases CAD risk among female Caucasian-Brazilians, irrespective of other CAD risk factors. In addition, *192GlnGln PON1* homozygotes show higher triglyceride and lower HDL-cholesterol levels in male Caucasian-Brazilians. No associations were detected among African-Brazilians.
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Keywords: African-Brazilians; coronary artery disease; paraoxonase; polymorphism.

Atherosclerosis is characterized by lipid deposition on the inner layer of the arterial wall. Oxidized low-density lipoprotein (LDL) is the major source of lipids for foam cell formation during atherogenesis and higher levels of LDL are associated with high oxidative stress conditions (1, 2). High-density lipoprotein (HDL) plays a key role in protecting LDL from oxidation, in part due to an esterase called paraoxonase 1 (PON1) associated with HDL particles that can hydrolyze specific oxidized lipids and lipoproteins (3, 4). Direct evidence of its role in atherogenesis has come from *PON1* and apolipoprotein E (*apoE*) gene double-knockout mice that presented higher lipid peroxidation levels and developed more atherosclerosis (5, 6).

PON1 is member of the paraoxonase family that includes three isozymes. Their genes are clustered on chromosome 7 q 21.3–22.1 (7). The *PON1* gene has two widely investigated polymorphisms, Met55Leu and Gln192Arg, which have previously been associated with LDL anti-oxidation activity (8) and coronary artery disease (CAD) risk among Caucasians and Asians in some but not all studies [reviewed in (9)].

The present study aimed to investigate Met55Leu and Gln192Arg *PON1* polymorphisms and their associations with angiographically assessed CAD and lipid levels in Caucasian- and African-Brazilians.

A total of 712 patients (437 Caucasian- and 275 African-Brazilians), who had undergone coronary angiography at Santa Izabel Hospital in Salvador, Bahia state, in Northeastern Brazil, as previously described (10), were studied. Angiography had been recommended due to symptoms related to CAD, major angina. Of these, 268 individuals presented no visible coronary lesion on angiography and were taken as controls. Among these controls, none presented acute myocardial infarction, cerebral vascular infarction or transient ischemic attack. The other 444 individuals presented at least one obstructive lesion of $\geq$50% and were taken as CAD cases, 194 of whom also presented a previous history of myocardial infarction con-

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firmed by ECG and/or cardiac enzymes in their medical records.

Patients provided a detailed medical history and underwent physical examination. Patients with blood pressure $\geq$140/90 mm Hg and/or taking anti-hypertensive medication were described as having hypertension. Diabetes mellitus was defined as fasting glucose level $\geq$7.0 mmol/L and/or when the patient was taking anti-diabetic medication. Smoking was defined as self-reported current or past smoking. Patients who had a first-degree relative who had had a myocardial infarct, sudden death and/or angina by the age of 55 years for male or 65 years for female were considered to have a positive early CAD family history. African- and Caucasian-Brazilians were classified according to skin pigmentation on the inner forearm and morphological facial characteristics, as previously reported and validated by classical genetic marks (11). All individuals provided written informed consent approved by the Hospital Ethics Committee.

Blood samples were drawn for DNA extraction and biochemical analysis from subjects who had fasted for at least 12 h. A salting out procedure method was used for DNA extraction as previously reported (12). The Met55Leu and Gln192Arg *PON1* polymorphisms were detected using a mismatched primer set in a multiplex PCR adapted from Motti et al. (13). The PCR product was digested with *Hin*fI and the fragment bands were viewed after acrylamide gel electrophoresis and ethidium bromide staining. Total cholesterol, HDL-cholesterol and triglyceride levels were measured by enzymatic methods using commercial kits (Wiener Lab, Rosario, Argentina) on an auto-analyzer. LDL-cholesterol levels were calculated using the Friedwald formula (14).

Allele frequencies were estimated by gene counting. The agreement of genotype frequencies with Hardy-Weinberg expectations was tested by a χ^2 goodness-of-fit test using Arlequin Program, version 2.000 (15). ANOVA or Student's t-test was used to compare quantitative variables between groups. The lipoprotein levels were log transformed to reach normal distribution before ANOVA or t-test analysis, but untransformed levels are presented in the Tables. Allele frequency differences between cases and controls were compared by Pearson χ^2 test using the PEPI program, version 4.0 (16). Odds ratio (OR) estimates and multivariate logistic regression were performed using SPSS version 10 (SPSS Inc., Chicago, IL, USA). A p-value of <0.05 was considered statistically significant.

Table 1 presents the clinical and demographic characteristics of CAD cases and controls. There was a higher prevalence of males, smokers, those with diabetes mellitus and early CAD family history, as well as those who had higher triglyceride and lower HDL-cholesterol levels among CAD cases than controls. Cases and controls were similar with respect to hypertension, total and LDL-cholesterol levels. However, in the same age range, CAD cases were somewhat older (55.5 years) than controls (52.3 years), and there was a lower frequency of African-Brazilians among cases compared to controls.

The Gln192Arg and Met55Leu *PON1* genotype frequencies differed between Caucasian- and African-Brazilians. The *192ArgArg* homozygous frequency was higher, while the *55MetMet* genotype frequency was lower among African-Brazilians (28% and 16.7%, respectively) compared to Caucasian-Brazilians (17.8%, $p=0.002$ and 26.3%, $p=0.010$, respectively). Among the control group, the genotype frequencies were in accordance with those expected by Hardy-Weinberg equilibrium, except for the Met55Leu variant in Caucasian-Brazilian males, which showed deviation from equilibrium due to a lower heterozygous frequency than expected.

Table 2 presents Met55Leu and Gln192Arg *PON1* genotype frequencies in CAD cases and controls according to ethnic and gender groups. In Caucasian-Brazilians, the *55LeuLeu* genotype frequency was higher in CAD cases (51%) compared to controls (30.3%; $p=0.022$) in females, but not males. Among African-Brazilians, the Met55Leu polymorphism was not associated with CAD. The Gln192Arg *PON1* genotype frequencies were not associated with CAD in African- and Caucasian-Brazilians for both male and female subjects (Table 2).

Table 3 presents the triglyceride and HDL-cholesterol levels among Gln192Arg *PON1* genotypes in the total sample of cases and controls according to gender and ethnic group. Among Caucasian-Brazilians, *192GlnGln PON1* homozygotes presented the highest

Table 1 Clinical and demographic characteristics of CAD cases and controls.

Variable	CAD cases	Controls	p
Number	444	268	–
Age, years	55.5±7.0	52.3±8.2	<0.001
Female/male	149/295	147/121	<0.001
Diabetes mellitus, yes/no	128/316	28/240	<0.001
Hypertension, yes/no	324/120	179/89	0.095
Current or past smoking, yes/no	254/190	117/151	0.001
Early CAD family history, yes/no	165/279	68/200	0.002
Total cholesterol, mmol/L	4.89±1.34	4.85±1.12	0.972
LDL-cholesterol, mmol/L	3.26±1.18	3.28±1.02	0.492
HDL-cholesterol, mmol/L	0.73±0.20	0.80±0.22	<0.001
Triglycerides, mmol/L	1.95±1.24	1.67±0.98	<0.001
Cholesterol-lowering medication, yes/no	153/297	45/223	<0.001
African-Brazilians/Caucasian-Brazilians	148/296	127/141	<0.001

Table 2 Met55Leu and Gln192Arg *PON1* frequencies in Caucasian- and African-Brazilians CAD cases and controls according to gender.

	Male				Female			
Caucasian-Brazilians	CAD cases	Controls	χ^2	p	CAD cases	Controls	χ^2	p
Met55Leu genotypes	n=196	n=65			n=100	n=76		
MetMet	56 (28.6%)	21 (32.3%)			18 (18%)	20 (26.3%)		
MetLeu	64 (32.7%)	15 (23.1%)			31 (31%)	33 (43.4%)		
LeuLeu	76 (38.7%)	29 (44.6%)			51 (51%)	23 (30.3%)		
			2.124	0.346			7.632	0.022
OR*=0.786; 95% CI 0.446–1.386; p=0.406					OR*=2.398; 95% CI 1.281–4.490; p=0.006			
Gln192Arg genotypes								
GlnGln	92 (46.9%)	32 (49.2%)			36 (36%)	39 (51.3%)		
GlnArg	68 (34.7%)	21 (32.3%)			45 (45%)	26 (34.2%)		
ArgArg	36 (18.4%)	12 (18.5%)			19 (19%)	11 (14.5%)		
			0.136	0.934			4.142	0.126
OR**=0.994; 95% CI=0.482–2.049; p=0.986					OR**=1.386; 95% CI 0.616–3.119; p=0.430			
African-Brazilians	CAD cases	Controls	χ^2	p	CAD cases	Controls	χ^2	p
Met55Leu genotypes	n=99	n=56			n=49	n=71		
MetMet	19 (19.2%)	11 (19.6%)			6 (12.2%)	10 (14.1%)		
MetLeu	34 (34.3%)	20 (35.8%)			16 (32.7%)	26 (36.6%)		
LeuLeu	46 (46.5%)	25 (44.6%)			27 (55.1%)	35 (49.3%)		
			0.049	0.976			0.393	0.822
OR*=1.076; 95% CI 0.557–2.079; p=0.827					OR*=1.262; 95% CI 0.608–2.620; p=0.532			
Gln192Arg genotypes								
GlnGln	45 (45.5%)	18 (32.1%)			13 (26.5%)	21 (29.6%)		
GlnArg	25 (25.3%)	21 (37.5%)			24 (49%)	31 (43.7%)		
ArgArg	29 (29.3%)	17 (30.4%)			12 (24.5%)	19 (26.8%)		
			3.381	0.184			0.332	0.847
OR**=0.950; 95% CI 0.465–1.944; p=0.889					OR**=0.888; 95% CI 0.384–2.049; p=0.780			

OR, odds ratio; CI, confidence interval. **55LeuLeu* genotype vs. *55Met* carriers, ** *192ArgArg* genotype vs. *192Gln* carriers.

triglyceride (p=0.012) and lowest HDL-cholesterol levels (p=0.035) in males, but not in females. In African-Brazilians, this polymorphism was not associated with differences in lipid levels. No other lipoprotein associations were detected with Gln192Arg *PON1* genotypes and no lipid association at all was observed for Leu55Met *PON1* genotypes (data not shown).

Table 4 shows the multivariate logistic regression analyses for CAD risk among Caucasian-Brazilians. *55LeuLeu PON1* homozygotes were at increased CAD risk (OR=2.852; p=0.003) and there was a significant interaction between this genotype and gender (OR=0.290; p=0.008). These effects were independent and corrected for other classic CAD risk factors included in the regression model. Gln192Arg *PON1* genotypes and other gene-smoking or gene-gender interactions were not significant CAD predictors. Among African-Brazilians, none of the *PON1* genotypes were significantly associated with CAD risk in the multivariate logistic regression, even after correcting for other CAD risk factors (data not shown).

This investigation of the effects of *PON1* gene polymorphisms on angiographically assessed CAD in a multi-ethnic Brazilian population has shown that *55LeuLeu PON1* homozygotes were at greater CAD risk among Caucasian-Brazilians, irrespective of lipoprotein levels and other classic CAD risk factors, as

Table 3 Triglyceride and HDL-cholesterol levels among Gln192Arg *PON1* genotypes in Caucasian- and African-Brazilians according to gender.

	Caucasian-Brazilians		African-Brazilians	
	Female (n=176)	Male (n=261)	Female (n=120)	Male (n=155)
Triglycerides, mmol/L				
GlnGln	1.84±1.68	2.00±1.09	1.53±0.91	1.93±1.55
GlnArg	2.13±1.20	1.64±0.98	1.62±0.63	1.93±1.22
ArgArg	1.83±1.02	1.79±0.80	1.48±0.71	2.03±1.03
	p=0.128	p=0.012	p=0.377	p=0.576
HDL-cholesterol, mmol/L				
GlnGln	0.81±0.20	0.67±0.19	0.83±0.25	0.71±0.17
GlnArg	0.80±0.17	0.71±0.22	0.84±0.20	0.70±0.23
ArgArg	0.89±0.29	0.74±0.17	0.86±0.22	0.75±0.19
	p=0.300	p=0.035	p=0.565	p=0.423

Results are for cases and controls combined.

Table 4 Multiple logistic regression for CAD risk in Caucasian-Brazilians.

Variable	β	SE	Wald	OR	95% CI		p
					Lower	Upper	
Age	0.065	0.016	16.488	1.068	1.034	1.102	<0.001
Male gender	1.427	0.319	20.020	4.165	2.230	7.782	<0.001
Early CAD history	0.555	0.247	5.025	1.742	1.072	2.829	0.025
Diabetes mellitus	1.376	0.361	14.520	3.959	1.951	8.036	<0.001
Hypertension	0.453	0.256	3.136	1.573	0.953	2.596	0.077
Smoking	0.622	0.238	6.830	1.862	1.168	2.969	0.009
HDL-cholesterol	–1.353	0.603	5.044	0.258	0.079	0.842	0.025
LDL-cholesterol	0.061	0.115	0.286	1.063	0.849	1.332	0.593
Triglycerides	0.004	0.101	0.001	1.004	0.823	1.223	0.972
55Leu PON1 homozygous	1.048	0.351	8.921	2.852	1.434	5.673	0.003
55Leu PON1 homozygous×gender	–1.239	0.470	6.932	0.290	0.115	0.729	0.008

Variables included in the model: age, gender, diabetes mellitus, hypertension, smoking (past and current), early CAD family history, HDL-cholesterol, LDL-cholesterol and triglyceride levels (mmol/L), *55Leu PON1* homozygous state, *55Leu PON1* homozygous state×gender, *55Leu PON1* homozygous state×smoking, *192Arg PON1* homozygous state, *192Arg PON1* homozygous state×gender, and *192Arg PON1* homozygous state×smoking.

shown by the multivariate logistic regression analysis. The present study corroborates a previous investigation in Southern Caucasian-Brazilians (17); however, it further demonstrates that the *55LeuLeu* effect on CAD risk is influenced by gender in our population.

The Met55Leu and Gln192Arg *PON1* polymorphism frequencies among African- and Caucasian-Brazilians were similar to those previously reported in related ethnic Brazilian populations from the Southern region (17, 18), except for the *55Met* allele frequency, which was higher than those previously reported for African-Brazilians from this region (18). Ancestral African origins vary among African-Brazilians according to region (19), which could explain this difference. The Met55Leu genotype frequencies were not those expected by Hardy-Weinberg equilibrium in Caucasian-Brazilian males. It is unlikely that genotyping errors could be the cause of this deviation, since we confirmed the genotyping, and the deviation is due to an excess of homozygous and not heterozygous genotypes, as expected for this type of error. It is more likely that the association of the *55LeuLeu* homozygous genotype with CAD could lead to deviation of the homozygous genotype frequencies from Hardy-Weinberg equilibrium.

The only previous study (20) of Gln192Arg and Met55Leu *PON1* polymorphism effects on angiographically assessed CAD in African-Americans showed no genotype association with this disease. Among Caucasian and Asian populations, *192Arg* and/or *55Leu PON1* variants were positively associated with increased CAD risk in some (17, 21–25), but not all studies (26–28). The present report corroborates a positive association between *55LeuLeu PON1* and CAD risk in females, supporting a gender-specific interaction. The exact cause of this *PON1* gender-specific effect on CAD is not known, although a *PON1* genotype effect on carotid atherosclerosis in females, but not in males, has previously been demonstrated (29). It is possible that hormonal differences between genders could influence lipoprotein oxidation and therefore the *PON1* effect on atherosclerosis (30). Smoking did not seem to be the cause of this genotype gender-specific effect, since the *55LeuLeu*× smoking interaction was not a significant CAD predictor in the multivariate logistic model. Furthermore, as HDL and PON1 synthesis are highly correlated (31) and this HDL production is clearly influenced by steroid hormones (32), it is possible that the *PON1* genotype gender-specific effects could be, at least in part, indirectly explained by hormonal influences on HDL synthesis.

Although not associated with CAD, *192GlnGln PON1* homozygotes presented lower HDL-cholesterol and higher triglyceride levels among Caucasian-Brazilian males, as previously reported in Caucasian-Americans (29). The adverse lipoprotein profile for the *192GlnGln PON1* genotype contrasts with its high enzyme activity in preventing LDL oxidation (8), which could be a possible explanation for the lack of association with CAD shown in the present study.

In summary, this study on angiographically assessed CAD in a multi-ethnic Brazilian sample demonstrates that *55LeuLeu PON1* homozygotes are at increased CAD risk among Caucasian- but not African-Brazilians, and this effect is influenced by gender, being more significant among females. Subjects homozygous for *192GlnGln PON1* had lower HDL-cholesterol and higher triglyceride levels in Caucasian-Brazilian males, but this genotype was not associated with CAD risk.

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