

Paraoxonase polymorphisms are associated with nitrate levels and vascular response in young adults with myocardial infarction

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Abstract

Objectives: To investigate the relationship between *PON1* and *PON2* polymorphisms and vascular reactivity and atherosclerotic biomarkers in young adults with acute myocardial infarction (AMI). **Design:** Young adults (<40 y) with AMI (n=113) and without AMI (control group, n=99) were selected in São Paulo city. **Methods:** Blood samples were collected for DNA extraction and total nitrate, inflammatory markers and serum lipids. Flow-mediated vasodilatation (FMV) induced or not by nitrate (FMN) was also evaluated. *PON1* (L55M, Q192R) and *PON2* (S311C) polymorphisms were analyzed by PCR-RFLP. **Results:** *PON1* L55M was associated with increased risk for AMI in young adults (OR: 1.589; CI 95%: 1.077–2.343). AMI patients carrying *PON1* 55LL genotype had increased serum nitrate and reduced FMV and FMN (p<0.05). *PON1* 55LL/192QQ haplotype was associated with increased serum nitrate (OR:5.25, CI 95%: 1.28–21.58, p=0.022) and FMN (OR:5.71, CI 95%: 1.22–26.88, p=0.027). In the control group, *PON1* 55LL genotype carriers had higher total cholesterol, LDL-C, VLDL-C, triglycerides and apoB than those carrying 55M allele (LM+MM genotypes) (p<0.05). Moreover, the *PON1* 55LL/192QR+RR haplotype was associated with increased total cholesterol and LDL-C (OR: 3.05, CI 95%: 1.01–9.20, p=0.048) and increased apoB (OR: 3.91, CI 95%: 1.25–12.18, p=0.019) in controls. **Conclusion:** *PON1* variants seem to play an important role in vascular response and serum nitrate homeostasis in AMI patients and in serum lipids variations in young adults.

Key words: Paraoxonase polymorphism; Myocardial infarction; Vascular response; Atherosclerosis

Introduction

Paraoxonase 1 (PON1) is a high-density lipoprotein (HDL)-associated enzyme that protects both HDL and low-density lipoprotein (LDL) from oxidation and preserves their function¹. *PON1* hydrolyzes oxidized phos-

pholipids from LDL and other lipoproteins, decreasing cytotoxicity and proinflammatory phospholipid activity and reducing the macrophage foam cell formation and atherosclerosis process as well². Paraoxonase 3 (PON3) also is part of HDL whereas paraoxonase 2 (PON2) is

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found in several tissues, such as endothelial and smooth muscle cells, and macrophages^{2,3}. *PON2* isoform has also antioxidant activity in preventing LDL oxidation².

Single nucleotide polymorphisms (SNPs) of the *PON1* (L55M and Q192R) and *PON2* (S311C) genes have shown to be related to increased risk for coronary artery disease (CAD) and other atherosclerotic diseases in several populations⁴⁻⁷. Moreover, relationships between *PON1* and *PON2* variants and acute myocardial infarction (AMI) were also demonstrated in Asian, European and American populations⁸⁻¹¹. These polymorphisms were suggested to influence paraoxonase activity and HDL binding affinity that reduces its protective role against lipoprotein oxidation^{12,13}.

We investigated whether *PON1* and *PON2* SNPs influence vascular reactivity and atherosclerotic biomarkers in young adults with AMI.

Patients and methods

Study population

Young adults (up to 40 years) with AMI (n=113) and without AMI (control group, n=99) were selected at the Dante Pazzanese Institute of Cardiology (Instituto Dante Pazzanese de Cardiologia, IDPC, São Paulo, SP, Brazil). AMI patients underwent coronary angiography in order to determine the reduction of the internal diameter of the coronary arteries as previously described¹⁴. All participants were evaluated for the presence of risk factors for CAD, such as diabetes, hypertension, dyslipidemia, obesity, smoking habit, family history of CAD.

Individuals with chronic renal insufficiency, hepatic insufficiency, collagenosis, inflammatory disease, instable angina, hypothyroidism, hyperthyroidism, familial hypercholesterolemia, peripheral vascular disease, myocardial pathology, pericardial pathology, congenital diseases, hematological disease, cancer, and using contraceptives, were not included in the study.

The study protocol was approved by the Ethics Committee of IDPC and Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo, SP, Brazil (Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, Brasil). Informed consent was obtained from each participant.

Flow-mediated vasodilatation

Flow-mediated vasodilatation (FMV) was measured by ultrasound of the brachial artery using a GE System Five with 7.5–12.0 MHz linear transducer, as previously described¹⁴. Participants were informed to be resting for 12 hours beforehand, without physical activities, smoking, in-

gesting vitamin C, and drinking coffee and commercial water with more than 1.1 mg nitrate. The women were tested during the low-estrogen level period. Statins were washed out for four weeks and antihypertensive medication was suspended 24 hours before the test. Vasodilatation was induced by a 5-min period of ischemia (arterial occlusion cuff 2 cm up to the antecubital fossa, with more than 50 mmHg the arterial systolic pressure). Flow rate and diameter of the brachial artery was measured each 20 s to obtain the higher vasodilatation data to calculate FMV. The endothelium-independent vasodilatation was performed at least 10 min after FMV resting time. Flow-mediated vasodilatation induced by nitrate (FMN) was measured five min after sublingual administration of 0.5 mg isosorbide dinitrate. Vessel diameters after reactive hyperemia and nitrate administration were compared with resting (basal) diameters and expressed as a percentage of the average lumen diameter at rest, as previously described¹⁴. Reference values for FMV and FMN were considered lower than 10% of basal value.

Blood sampling and laboratory analyses

Blood samples were collected after 12 h fast for DNA extraction and biochemical determinations. Glucose, total cholesterol, HDL cholesterol, triacylglycerols were determined in serum by routine enzymatic procedures using a fully automated system Hitachi mod 912 (Roche Diagnostic, São Paulo). LDL and VLDL cholesterol were calculated using the Friedewald formula when the triglyceride concentration did not exceed 4.52 mmol/L. Serum apolipoprotein (apo) B and apo A1 were determined by immunonephelometry (Behring, Marburg, Germany).

Serum C-reactive protein (CRP) was measured by turbidimetric immunoassay (Roche Diagnostics, USA). Plasma PAI-1 and fibrinogen were determined by ELISA and spectrophotometry (Electra mod 1400C), respectively. Serum total nitrate was determined using Nitric Oxide Analyzer mod 280 (Sievers Instruments).

DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood leukocytes by salting-out procedure¹⁵. *PON1* L55M (rs662), *PON1* Q192R (rs854560) and *PON2* S311C (rs7493) polymorphic regions were detected after DNA amplification by polymerase chain reaction (PCR). The primer sequences were selected for *PON1* L55M (forward: 5'-CACAATTTGTCTTTAAACGATGA-3' and reverse 5'-AGGTGTGATAAAGAAATGGATCC-3'), *PON1* Q192R (forward: 5'-CCATTATAGCTAGCACGAAGGC-3' and reverse 5'-GCCATCGGGTGAAATGTTGA-3'), and *PON2* S311C (forward: 5'-GTTAAGTTATCGCACTTTGATGC-3'

Table 1. Demographic, atherosclerotic biomarkers and vascular reactivity in AMI and control groups/

Variables	AMI n=113	Controls n=99	p-value
Age, years	34±5	35±9	0.646
Ethnicity (non-African descent)	66.3%	71.9%	0.598
Gender (men)	68.1%	73.3%	0.394
Diabetes mellitus	9%	0%	0.001
Dyslipidemia	50%	30%	0.003
Hypertension	47%	1%	<0.001
Obesity	31%	6%	<0.001
Cigarette smoking	84%	7%	<0.001
Family history of CAD	48%	13%	<0.001
Body mass index, kg/m ²	27.5±4.3	23.7±3.4	<0.001
FMV, %	12±8	17±9	<0.001
FMN, %	18±7	18±6	0.650
Nitrate, µmol/L	19.3±9.2	17.9±10.3	0.125
CRP, ng/mL	0.37±0.58	0.49±1.73	0.760
Fibrinogen, g/L	3.19±0.84	2.68±0.69	<0.001
PAI-1, U/mL	18.3±11.1	7.8±7.0	<0.001
Glucose, mmol/L	6.13±2.86	5.07±0.54	<0.001
Total cholesterol, mmol/L	5.49±1.35	4.79±1.11	<0.001
HDL-c, mmol/L	1.16±0.34	1.45±0.39	<0.001
LDL-c, mmol/L	3.39±1.09	2.85±0.95	<0.001
VLDL-c, mmol/L	0.88±0.47	0.52±0.31	<0.001
Triglycerides, mmol/L	2.06±1.18	1.07±0.53	<0.001
Apolipoprotein AI, g/L	1.25±0.26	1.47±0.25	<0.001
Apolipoprotein B, g/L	1.07±0.27	0.85±0.23	<0.001

Values are presented as mean±SD compared by Student's t-test. Categorical variables were compared by chi-square test. AMI=acute myocardium infarction; AD=coronary artery disease; CRP=ultrasensitive C protein reactive; FMN=flow-mediated vasodilatation induced by nitrate; FMV=flow-mediated vasodilatation; LDL-C=low-density lipoprotein cholesterol; HDL-C=high-density lipoprotein cholesterol; PAI-1=plasminogen activator inhibitor-1; VLDL-C=very low-density lipoprotein cholesterol.

and reverse 5'-CCTTAATCAGTGTGCATTGTGG-3') polymorphisms.

Genomic DNA (100–200 ng) was amplified in 50 µl reaction mixture containing 200 nM primers (Invitrogen Corporation, CA, USA), 200 µM dNTPs (GE Healthcare, Buckinghamshire, England), 1.0 U *Taq* DNA polymerase and reaction buffer (75 mM Tris-HCl [pH 9.0], 50 mM KCl, 2.0 mM MgCl₂, 20 mM (NH₄)₂SO₄) (Biotools, Madrid, Spain). Then the DNA was denatured for 3 min at 98°C, the reaction mixture was subjected to 30 cycles at 95°C for 1 min, 46°C (L55M) or 55°C (Q192R) or 52°C (S311C) for 2 min, and 72°C for 1 min, using a PTC-200 Thermal Cycler (MJ Research Inc., MA, USA).

The *PON1* (L55M and Q192R) and *PON2* (S311C) polymorphisms were detected by digesting the PCR-amplified product with the *Nla*III, *Mbo*I and *Dde*I endo-

nucleases, respectively, followed by size fractionation using 2% agarose gel electrophoresis. Quality control was carried out by repeating randomly selected 20% of all samples.

Statistical analysis

Chi-square analysis was used to compare frequencies between categorical variables and also to test Hardy-Weinberg equilibrium (HWE). Continuous variables were compared by Student's t-test. Data without normal distribution were transformed into log₁₀. Logistic regression analysis was performed using SAS software program V. 6.12 to evaluate the relationship between *PON1* haplotypes and continuous variables. The relative risk and 95% confidence interval were estimated using the exposure odds ratio. The level of significance was considered 5%.

Table 2. Genotype and allele frequencies of *PON1* and *PON2* polymorphisms in AMI and control groups.

Polymorphisms	AMI	Controls	
<i>PON1</i> L55M			
LL	40.7% (46)	27.3% (27)	
LM	45.1% (51)	49.5% (49)	
MM	14.2% (16)	23.2% (23)	$\chi^2=5.340$; $p=0.069$
L allele	63.3%	52.0%	
M allele	36.7%	48.0%	$\chi^2=5.036$; $p=0.025$
<i>PON1</i> Q192R			
QQ	31.0% (35)	27.3% (27)	
QR	49.5% (56)	52.5% (52)	
RR	19.5% (22)	20.2% (20)	$\chi^2=0.353$; $p=0.838$
Q allele	55.8%	53.5%	
R allele	44.2%	46.5%	$\chi^2=0.129$; $p=0.719$
<i>PON2</i> S311C			
SS	61.1% (69)	53.5% (53)	
SC	36.3% (41)	40.4% (40)	
CC	2.6% (3)	6.1% (6)	$\chi^2=2.196$; $p=0.334$
S Allele	79.2%	73.7%	
C Allele	20.8%	26.3%	$\chi^2=1.470$; $p=0.225$
<i>PON1</i> haplotypes			
LL/QQ	12% (13)	7% (7)	
LL/QR+RR	29% (32)	21% (20)	
LM+MM/QQ	20% (22)	19% (19)	
LM+MM/QR+RR	39% (44)	53% (52)	$\chi^2=4.665$; $p=0.198$

Number of individuals in parenthesis. AMI; acute myocardium infarction.

Results

The demographic, atherosclerotic biomarkers and vascular reactivity of the AMI and control groups are summarized in Table 1. Mean age and the frequencies of men and ethnic backgrounds were similar in both AMI and control groups ($p>0.05$). The mean BMI value and frequencies of diabetes, dyslipidemia, hypertension, obesity, smoking habit and family history of CAD were higher in the AMI group than in controls ($p<0.003$).

FMV was lower and fibrinogen and PAI-1 plasma concentrations were higher in AMI individuals when compared to the controls ($p<0.001$). Total nitrate and CRP concentrations did not differ between AMI and control groups (Table 1). Serum concentrations of glucose, total cholesterol, LDL cholesterol, VLDL cholesterol, triglycerides and Apo B were higher in AMI patients than in the control group, whereas HDL cholesterol and Apo AI were lower in the AMI group ($p<0.001$).

PON1 and *PON2* genotype distributions in AMI and control groups were in HWE (data not shown). Allele analysis showed that AMI patients had a higher frequency for the 55L allele (63.3%) than the control group (52.0%, $p=0.025$) (Table 2). Moreover, homozygous 55LL

genotype was also more frequent in AMI (40.7%) than in controls (27.3%), although the statistical difference was borderline ($p=0.069$). These data are suggestive that the *PON1* 55L allele is associated with increased risk for AMI in young adults (OR: 1.589; CI 95%: 1.077–2.343). On the other hand, no relationship was found between *PON1* Q192R and *PON2* S311C SNPs or *PON1* haplotypes and AMI in this study.

Comparisons of the atherosclerotic biomarker values between *PON1* L55M genotypes carriers are shown in Table 3. In the AMI group, *PON1* 55LL genotype was associated with higher total nitrate ($p=0.024$). On the other hand, control individuals carrying *PON1* 55LL genotype have higher serum total cholesterol ($p=0.015$), LDL cholesterol ($p=0.028$), VLDL cholesterol ($p=0.025$), triglycerides ($p=0.011$), and apo B ($p=0.048$) than the *PON1* 55LM+MM genotypes carriers. No associations were found between *PON1* Q192R or *PON2* S311C variants and differences in atherosclerotic biomarkers in either AMI patients or control group (data not shown).

Vascular reactivity analysis showed that *PON1* 55LL genotype carriers have lower FMV and FMN than those carrying 55LM+MM genotypes (Figure 1A). Whereas,

Table 3. Atherosclerotic biomarkers according to PON1 L55M genotypes in AMI and control groups.

Variables		55LL	55LM + 55MM	p
Individuals	AMI	46	67	
	Control	27	72	
Nitrate, $\mu\text{mol/L}$	AMI	21.8 \pm 10.6	17.5 \pm 7.8	0.024
	Control	19.2 \pm 12.1	17.5 \pm 9.9	0.761
CRP, ng/mL	AMI	0.32 \pm 0.75	0.40 \pm 0.43	0.140
	Control	0.25 \pm 0.51	0.59 \pm 2.01	0.283
Fibrinogen, g/L	AMI	3.13 \pm 0.81	3.22 \pm 0.86	0.697
	Control	2.49 \pm 0.64	2.75 \pm 0.70	0.065
PAI-1, U/mL	AMI	17.2 \pm 10.4	19.0 \pm 11.1	0.640
	Control	6.9 \pm 5.5	7.8 \pm 7.2	0.984
Glucose, mmol/L	AMI	5.78 \pm 1.98	6.38 \pm 3.46	0.327
	Control	4.95 \pm 0.50	5.06 \pm 0.50	0.643
TC, mmol/L	AMI	5.31 \pm 1.09	5.59 \pm 1.53	0.368
	Control	5.25 \pm 1.22	4.61 \pm 1.01	0.015
HDL-c, mmol/L	AMI	1.14 \pm 0.39	1.19 \pm 0.31	0.076
	Control	1.42 \pm 0.36	1.45 \pm 0.39	0.686
LDL-c, mmol/L	AMI	3.37 \pm 1.04	3.44 \pm 1.14	0.793
	Control	3.24 \pm 1.14	2.69 \pm 0.85	0.028
VLDL-c, mmol/L	AMI	0.83 \pm 0.41	0.91 \pm 0.52	0.871
	Control	0.60 \pm 0.31	0.47 \pm 0.31	0.025
TG, mmol/L	AMI	1.89 \pm 0.97	2.16 \pm 1.30	0.587
	Control	1.30 \pm 0.66	0.98 \pm 0.43	0.011
Apo AI, g/L	AMI	1.20 \pm 0.25	1.30 \pm 0.27	0.061
	Control	1.45 \pm 0.22	1.47 \pm 0.26	0.732
Apo B, g/L	AMI	1.08 \pm 0.26	1.06 \pm 0.28	0.682
	Control	0.92 \pm 0.24	0.82 \pm 0.22	0.048

Values are presented as mean \pm SD and compared by Student's t-test performed on the log-transformed variables. AMI=acute myocardial infarction; CRP=ultrasensitive C-reactive protein; LDL-c=low-density lipoprotein cholesterol; HDL-C=high-density lipoprotein cholesterol; PAI-1=plasminogen activator inhibitor-1; VLDL-C=very low-density lipoprotein cholesterol.

PON1 Q192R and *PON2* S311C variants were not associated with differences in the FMV or FMN in the AMI group (Figures 1B, 1C).

Laboratory variables from AMI patients and controls were grouped in quartiles. Data in the lower quartile (Q25) for FMV (\leq 6.0%), FMN (\leq 13.0%), HDL cholesterol (\leq 1.19 mmol/L) and apo AI (\leq 1.27 g/L), and in the higher quartile (Q75) for the other variables were used to test their relationship with *PON1* haplotypes by logistic regression analysis.

In the AMI group, individuals carrying *PON1* LL/QQ haplotype had a 5 times higher risk for increased nitrate ($>$ 20.8 $\mu\text{mol/L}$) (OR: 5.25, CI 95%: 1.28-21.58, $p=0.022$) and decreased FMN (\leq 13.0%) (OR: 5.71, CI 95%: 1.22-26.88, $p=0.027$) in comparison with the other haplotypes (Table 4). On the other hand, there was not relationship between *PON1* haplotypes and altered serum lipid profile in AMI individuals (data not shown).

In the control group, *PON1* LL/QR+RR haplotype carriers had a higher risk for increased serum total cholesterol (OR: 3.05, CI 95%: 1.01-9.20, $p=0.048$), LDL cholesterol (OR: 3.05, CI 95%: 1.01-9.20, $p=0.048$) and Apo B levels (OR: 3.91, CI 95%: 1.25-12.18, $p=0.019$) when compared to the other haplotypes (Table 5). In addition, *PON1* LL/QQ haplotype was associated with high risk for increased Apo B (OR: 6.37, CI 95%: 1.21-33.52, $p=0.029$). However, no relationship was found between *PON1* haplotypes and other atherosclerotic biomarkers and vascular reactivity in the control group (data not shown).

Discussion

In this study, *PON1* 55L allele was associated with AMI, as it has been shown previously in another sample of Brazilian individuals¹⁶. It has been suggested that the *PON1* L55M variant can be considered a predictive mark-

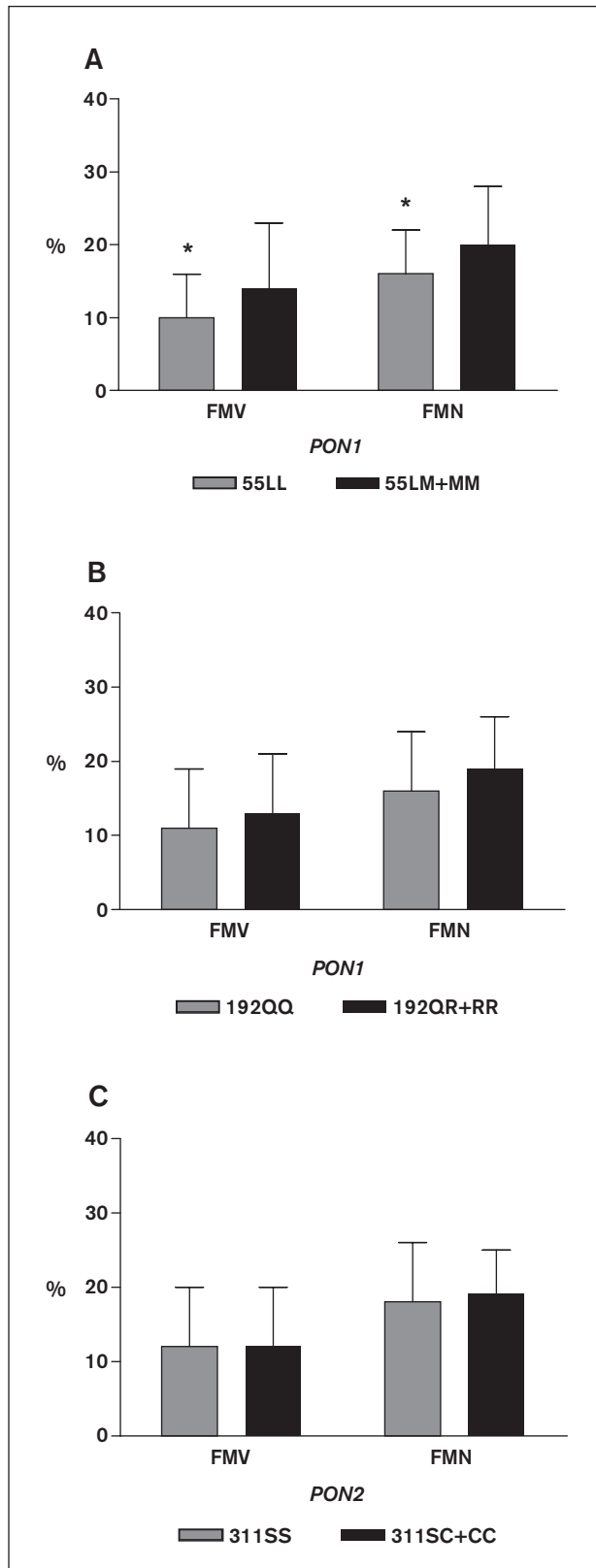


Figure 1. Flow-mediated vasodilatation (FMV) and flow-mediated by nitrate (FMN) and in AMI patients carrying *PON1* L55M (A); *PON1* Q192R (B) and *PON2* S311C (C) genotypes. Values are presented as mean \pm SD were compared by Student's t-test ($p < 0.05$).

er of protection against CAD¹⁶. On the other hand, *PON1* Q192R and *PON2* S311C variants were not related to AMI in our sample. In accordance with these results, the *PON1* Q192R variant was not considered a predictor of stenosis or myocardial infarction in patients with CAD¹⁶ and ischemic heart disease¹⁷. The *PON1* Q192R variant also did not play a role in the modulation of endothelial vasomotor function in the coronary circulation of patients with coronary spastic angina¹⁸. This variant was also not associated with CAD and myocardial infarction in a general population of Dutch women¹⁹. Even though gender, smoking habit and other CAD-risk related variables have been suggested to interact with *PON1* and *PON2* variants to enhance risk for CAD or AMI²⁰⁻²³, we did not find a relationship between these variables and *PON1* and *PON2* polymorphisms in our study.

It is noteworthy that *PON1* L55M SNP influenced nitrate levels and flow-mediated vasodilatation in AMI patients. Moreover, increased nitrate and reduced flow-mediated by nitrate were strongly associated with *PON1* LL/QQ haplotype. The relationship between *PON1* variants and vascular reactivity may result from their effects on plasma paraoxonase activity.

PON1 L55M and Q192R SNPs have shown to affect paraoxonase activity in plasma^{24,25}. In addition, *PON1* activity was shown to be positively correlated with endothelium-dependent vasodilatation in patients with cardiac syndrome X²⁶ and with slow coronary flow²⁷. Therefore, it is conceivable that AMI patients carrying both *PON1* 55LL and 192QQ genotypes had lower plasma paraoxonase activity, which contributes to the enhanced oxidative stress and impaired endothelial functions as shown by the reduced flow-mediated vasodilatation.

We demonstrated that *PON1* variants did not influence vascular reactivity in the control group. In accordance with this result, *PON1* Q192R polymorphism was not related with brachial reactivity to flow-induced vasodilatation in health adult individuals²⁸.

A relationship between *PON1* 55LL genotype and increased total cholesterol, LDL cholesterol, triglycerides and apo B in serum was found in individuals without AMI. A previous study has shown that *PON1* 55MM genotype was associated with lower triglyceride serum levels in individuals with CAD¹⁶. Although *PON1* Q192R variant has shown to be individually related to differences in serum lipids^{23,29}, our results are suggestive that the effects on lipid metabolism result from the interaction between 55L and 192Q alleles in the non-AMI group. Therefore, *PON1* variants may play an important role in lipid metabolism probably through their direct effect on paraoxonase activity in plasma as previously suggested³⁰. On the other hand,

Table 4. Logistic regression analysis of the variables associated with *PON1* haplotypes in AMI patients.

Dependent variable	Independent variable	Comparisons	p	OR	CI 95%
FMV ≤6.0 %	L55M/Q192R haplotype	LM+MM / QR+RR (ref.) (40)	–	1.00	–
		LM+MM / QQ (19)	0.748	1.23	0.35–4.35
		LL / QR+RR (30)	0.935	1.05	0.34–3.23
		LL / QQ (11)	0.355	1.97	0.47–8.27
FMN ≤13.0%	L55M/Q192R haplotype	LM+MM / QR+RR (ref.) (39)	–	1.00	–
		LM+MM / QQ (14)	0.776	1.25	0.27–5.68
		LL / QR+RR (29)	0.534	1.46	0.45–4.73
		LL / QQ (9)	0.027	5.71	1.22–26.88
Nitrate > 20.8 μmol/L	L55M/Q192R haplotype	LM+MM / QR+RR (ref.) (43)	–	1.00	–
		LM+MM / QQ (19)	0.431	0.52	0.10–2.69
		LL / QR+RR (31)	0.296	1.79	0.60–5.33
		LL / QQ (11)	0.022	5.25	1.28–21.58
CRP >0.50 ng/mL	L55M/Q192R haplotype	LM+MM / QR+RR (ref.) (44)	–	1.00	–
		LM+MM / QQ (21)	0.078	0.29	0.07–1.15
		LL / QR+RR (32)	0.052	0.32	0.10–1.01
		LL / QQ (13)	0.077	0.15	0.02–1.23
Fibrinogen >3.58 g/L	L55M/Q192R haplotype	LM+MM / QR+RR (ref.) (43)	–	1.00	–
		LM+MM / QQ (21)	0.891	0.92	0.29–2.91
		LL / QR+RR (32)	0.149	0.43	0.14–1.36
		LL / QQ (13)	0.131	0.19	0.02–1.64
PAI-1 >26.6 U/mL	L55M/Q192R haplotype	LM+MM / QR+RR(ref.) (43)	–	1.00	–
		LM+MM / QQ (21)	0.474	0.65	0.20–2.13
		LL / QR+RR (31)	0.117	0.40	0.13–1.26
		LL / QQ (12)	0.618	0.69	0.16–2.96

Number of individuals in parenthesis. AMI=acute myocardium infarction; CRP=ultrasensitive C-reactive protein; FMN=flow-mediated by nitrate; FMV=flow-mediated vasodilatation; PAI-1=plasminogen activator inhibitor-1.

Table 5. Logistic regression analysis of the variables associated with *PON1* haplotypes in the control group.

Dependent variable	Independent variable	Comparisons	p	OR	CI 95%
TC >5.41 mmol/LI	L55M/Q192R Haplotype	LM+MM / QR+RR (ref.) (52)	–	1.00	–
		LM+MM / QQ (19)	0.953	0.00*	0.00–999.0
		LL / QR+RR (20)	0.048	3.05	1.01–9.20
		LL / QQ (7)	0.219	2.8	0.54–14.39
HDL-c ≤1.19 mmol/L	L55M/Q192R Haplotype	LM+MM / QR+RR (ref.) (52)	–	1.00	–
		LM+MM / QQ (19)	0.959	0.97	0.30–3.19
		LL / QR+RR (20)	0.868	0.91	0.28–2.95
		LL / QQ (7)	0.927	1.09	0.19–6.25
LDL-c >3.50 mmol/L	L55M/Q192R Haplotype	LM+MM / QR+RR (ref.) (52)	–	1.00	–
		LM+MM / QQ (19)	0.952	0.00*	0.00–999.0
		LL / QR+RR (20)	0.048	3.05	1.01–9.20
		LL / QQ (7)	0.055	4.97	0.97–25.57
VLDL-c >0.60 mmol/L	L55M/Q192R Haplotype	LM+MM / QR+RR (ref.) (52)	–	1.00	–
		LM+MM / QQ (19)	0.489	0.56	0.11–2.87
		LL / QR+RR (20)	0.112	2.57	0.80–8.26
		LL / QQ (7)	0.478	1.91	0.32–11.45
TG >1.19 mmol/L	L55M/Q192R haplotype	LM+MM / QR+RR (ref.) (52)	–	1.00	–
		LM+MM / QQ (19)	0.74	0.79	0.19–3.24
		LL / QR+RR (20)	0.164	2.26	0.72–7.13
		LL / QQ (7)	0.172	3.15	0.61–16.37
Apo AI ≤1.27 g/L	L55M/Q192R haplotype	LM+MM / QR+RR (ref.) (52)	–	1.00	–
		LM+MM / QQ (19)	0.616	0.72	0.21–2.56
		LL / QR+RR (20)	0.868	0.91	0.28–2.95
		LL / QQ (7)	0.927	1.09	0.19–6.25
Apo B >0.98 g/L	L55M/Q192R haplotype	LM+MM / QR+RR (ref.) (52)	–	1.00	–
		LM+MM / QQ (19)	0.224	0.27	0.03–2.25
		LL / QR+RR (20)	0.019	3.91	1.25–12.18
		LL / QQ (7)	0.029	6.37	1.21–33.52

Number of individuals in parenthesis. Apo AI=apolipoprotein AI; Apo B=apolipoprotein B; HDL-c=high-density lipoprotein cholesterol; LDL-C=low-density lipoprotein cholesterol; TC=total cholesterol; TG=triglycerides; VLDL-C=very low-density lipoprotein cholesterol.

PON2 variant seems to play a less important role in determining serum lipid variations in our sample.

In AMI patients, *PON1* and *PON2* SNPs were not related to differences in serum lipid profile. On the other hand, *PON1* 192QQ genotype was associated with reduced HDL cholesterol and increased triglycerides in Caucasian-Brazilian males with CAD but not in females²³. As mentioned before, the relationship between Q192R variant and serum lipids in CAD patients seems to be dependent on gender but we did not find this association in this study.

In conclusion, *PON1* variants seem to play an important role in vascular response and serum nitrate homeostasis in AMI patients and in serum lipid variations in young adults.

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