



BASIC RESEARCH

## Paraoxonase 1 gene polymorphisms and enzyme activities in coronary artery disease and its relationship to serum lipids and glycemia



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### KEYWORDS

Coronary artery disease;  
Paraoxonase;  
Genetic polymorphisms;  
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Lipid profile;  
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### Abstract

**Objectives:** Oxidative stress and inflammation are important processes in development of atherosclerosis. Paraoxonase 1 (PON1) is a bioscavenger enzyme associated with inflammation and oxidative stress. We evaluate the association of two single nucleotide polymorphisms in PON1 gene, and enzyme activities with lipid profile and glycemia.

**Methods:** This case–control study consisted of 126 patients with coronary artery disease (CAD) and 203 healthy controls. PON Q192R and L55M polymorphisms were detected by real-time PCR. Paraoxonase and arylesterase activities were determined spectrophotometrically. Blood glucose, cholesterol, triglycerides, HDL, and LDL were measured.

**Results:** PON1 QR192 polymorphism had a major effect on paraoxonase but no effect on arylesterase serum activities. Paraoxonase activity was higher in RR genotype and lowest in QQ genotype. Paraoxonase and arylesterase activities were higher in LL and lower in MM genotypes of PON1 LM55 polymorphism. RQ and LM variants showed intermediate activities between respective homozygous. Elevated concentrations of triglycerides in cases correlate with QQ variant or the presence of M allele. Glucose levels were elevated in cases with QQ variant or with the presence of M allele. Cholesterol and LDL did not show variations in control and cases with any variant of both polymorphisms. HDL is lower in cases with respect to controls independently of genotypes. All differences were significant with  $p < 0.05$ .

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**PALABRAS CLAVE**

Enfermedad coronaria;  
 Paraoxonasa;  
 Polimorfismos genéticos;  
 Actividades enzimáticas;  
 Perfil lipídico;  
 Glucemia;  
 Argentina

**Conclusiones:** Our results confirm the relationship between variations in PON1 activities and lipid metabolism, and showed that genetically programmed low PON1 activities would have certain responsibility in the increase in glycemia and concomitantly the aggravation of atherosclerotic disease.

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### Polimorfismos en el gen de la paraoxonasa 1 y sus actividades enzimáticas en la enfermedad coronaria. Su relación con el perfil lipídico y la glucemia

**Resumen**

**Objetivos:** La enzima paraoxonasa 1 (PON1), está asociada con el estrés oxidativo y la inflamación, procesos importantes en el desarrollo de la aterosclerosis. Evaluamos la asociación de 2 polimorfismos de un solo nucleótido en el gen PON1 y sus actividades enzimáticas con el perfil lipídico y la glucemia.

**Métodos:** Estudio caso-control en 126 pacientes con enfermedad coronaria y 203 controles sanos. Los polimorfismos PON Q192R y L55M fueron detectados por PCR en tiempo real y las actividades de paraoxonasa y arilesterasa por espectrofotometría. Se midieron glucemia, colesterol, triglicéridos, HDL y LDL.

**Resultados:** El polimorfismo PON1 QR192 afectó la actividad de paraoxonasa pero no la de arilesterasa. La actividad de paraoxonasa fue mayor en el genotipo RR y menor en QQ. Ambas actividades fueron mayores en el genotipo LL y menores en MM del polimorfismo PON1 LM55. Las variantes RQ y LM mostraron actividades intermedias entre los respectivos homocigotos. Concentraciones elevadas de triglicéridos en los casos correlacionaron con la variante QQ o la presencia del alelo M. Los niveles de glucosa fueron elevados en los casos QQ o con la presencia del alelo M. El colesterol y el LDL no variaron ni en los casos ni en los controles con ambos polimorfismos. El HDL fue menor en los casos respecto de los controles, independientemente del genotipo.

**Conclusiones:** Los resultados confirman la relación entre las variaciones en las actividades de PON1 y el metabolismo lipídico y mostraron que las bajas actividades de PON1 genéticamente programadas tendrían cierta responsabilidad en el aumento de la glucemia y, concomitantemente, en la agravación de la enfermedad aterosclerótica.

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**Introduction**

High-density lipoproteins (HDL) have received increasing attention because they have been shown to retard the oxidation of LDL. The HDL-associated enzyme, paraoxonase 1 (PON1), is one of the enzymes located on HDL particles responsible for preventing the accumulation of lipid peroxides on LDL.<sup>1-4</sup> The anti-atherogenic properties of HDL are mainly related to reverse cholesterol transport, stabilization of atherosclerotic plaque, and anti-inflammatory and antioxidant effects.<sup>5,6</sup> Apolipoprotein A-I (apoA-I) accounts for approximately 70% of the total protein mass of HDL, and the remaining protein components mainly include apoA-II, apoC, apoA-IV, and paraoxonase (PON).<sup>7</sup> PON1 has been shown to effectively hydrolyze the oxidized phospholipids present in LDL, thus retarding the oxidation of these lipoproteins, attenuating their pro-inflammatory effects and protecting vessels from atherosclerosis progression.<sup>2,8,9</sup> Moreover Aviram et al.,<sup>3</sup> have shown that PON1 also reduces HDL

lipid oxidation. Serum PON1 activity and concentration are decreased in subjects who have had a myocardial infarction compared with controls<sup>10</sup> and low serum PON1 activity represent an independent risk factor for coronary events.<sup>8</sup> Other diseases associated with alterations in circulating PON1 concentrations are Alzheimer's disease, chronic renal failure, chronic liver impairment, and type I and type II diabetes mellitus.<sup>11,12</sup> PON1, arylalkylphosphatase (EC 3.1.8.1) is a Ca<sup>2+</sup>-dependent serum esterase synthesized mainly by the liver<sup>13</sup> catalyzes the hydrolysis of many highly toxic xenobiotics.<sup>14</sup> PON1 gene is localized at q21-q22 on the long arm of chromosome 7 in humans.<sup>15</sup> Genetic variations of the PON1 gene modify serum PON1 properties<sup>15,16</sup> affecting its catalytic efficiency. The most studied polymorphisms of the PON1 gene are the glutamine to arginine substitution (Q/R) at codon 192 and the leucine to methionine substitution (L/M) at codon 55.<sup>16,17</sup> The activity of the polymorphism at position 192 (QR192) is substrate dependent. For instance, paraoxon and fenitroxon

are hydrolyzed faster by the R allele, while both isoforms hydrolyze chlorpyrifos-oxon and phenylacetate at approximately the same rate. The Q allele, on the other hand, hydrolyze more rapidly substrates such as soman, sarin and diazoxon.<sup>18</sup> Polymorphism at position 55 (LM55) has a much smaller, but significant, effect on PON1 activity than the QR192 polymorphism.<sup>19,20</sup> Therefore, there is a variation in the catalytic sites for different substrates and has been proposed that in humans, PON1 activity resides in the hydrolysis of the organophosphates paraoxon and phenylacetate.<sup>3</sup> Taking into account the growing importance of PON1 as a risk factor of atherosclerosis, the purpose of this study was to evaluate the association of two single nucleotide polymorphisms in the PON1 gene with the serum activities of paraoxonase and arylesterase and the risk factors associated with atherosclerosis progression, the lipid profile and glycemia, in non diabetics subjects with a cardiovascular event (cases) and controls from the city of Buenos Aires, Argentina to get a better understanding of the role of the PON1 Q192R and L55M polymorphisms in the development of atherosclerosis and coronary artery disease (CAD).

## Materials and methods

A case-control study was carried out for a period of one year between 2012 and 2013. A total of 329 subjects were included (randomized) from patients admitted and hospitalized to the Coronary Care Unit of the Cardiology Department with suspicion of acute coronary syndrome. Subjects who had liver diseases, renal diseases, thyroid diseases, diabetes and those on current therapy on antioxidant supplementation were excluded. The rest was intended to the case group if they met the following inclusion criteria: previous or current diagnosis of myocardial infarction or CAD. This diagnosis was made on the basis of the history, physical assessment, ECG patterns compatible with CAD (e.g.: presence of Q waves, ST elevation or depression, inverted T-waves, left bundle branch block, LBBB), biomarkers of myocardial injury (CPK that doubled the normal upper limit and CK-MB representing more than 6% of the amount of CPK or an absolute value greater than 28 according to the method used) or by coronary angiography that showed at least 75% obstruction of at least one coronary artery or 50% obstruction of the left main vessel. In all cases the final diagnosis was established according to the diagnostic algorithm in use and the medical judgment. Those individuals who did not meet the inclusion criteria were assigned to the control group. Based on the inclusion and exclusion criteria, a total number of 126 CAD cases and 203 age and sex matched controls were selected for the present study. Patients were consecutively selected as and when they presented to us. Both cases and controls were interviewed to obtain relevant data. Informed consents were obtained from all the subjects who were involved in the study. The patients and controls voluntarily participated in the study. Protocol was approved by the Ethical Committee of the University Hospital, UAI.

## Blood collection

Blood was taken by venipuncture in controls and cases after 12–14h of fasting in the morning. Blood was collected to

obtain serum samples and in EDTA containing tube to obtain the cellular sample. Serum was divided into 2 aliquots; one aliquot was used directly for the measurements of total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides and glucose. Another was kept in  $-80^{\circ}\text{C}$  until the analysis of serum PON-1 activities. Cellular samples for DNA extraction were kept at  $-20^{\circ}\text{C}$  up to use.

## Laboratory measurements

Biochemical measurement including blood glucose, cholesterol, triglycerides, HDL, and LDL were carried out using Metrolab 2300 auto-analyzer.

## Determination of PON genotype

Genomic DNA was isolated from nucleated blood cells using FlexiGene DNA Kit QIAGEN® GmbH (Hilden, Germany) following the manual instruction. The genotyping of the Q192R (rs662) and L55M (rs854560) polymorphisms in the PON1 gene were determined following PCR according to previously published protocols.<sup>15,16</sup> Each sample was genotyped twice and even three times if it was necessary.

## Paraoxonase and arylesterase activities

Serum activities of paraoxonase and arylesterase were measured spectrophotometrically using paraoxon and phenylacetate as substrates respectively according to protocols previously described.<sup>21,22</sup>

## Statistical analysis

Comparisons between groups were performed using a Student's 't' test or a one-way ANOVA for multiple comparisons. Allele frequencies were calculated by allele counting. The fitting of genetic distribution to the Hardy-Weinberg equilibrium (H-WE) was analyzed by using the Chi-square test. Odds ratios and 95% confidence intervals were calculated. Values are expressed as percentages or as means  $\pm$  SEM. All results were interpreted at an alpha level of 0.05. Data were analyzed using SPSS version 16.0 for Windows (SPSS Inc., Chicago, IL, USA).

## Results

### Characteristics of CAD patients and control subjects

Table 1 shows the demographic characteristics and biochemical values of the control and cases. Cases show significant higher values in triglycerides ( $p < 0.05$ ) and fasting glucose levels ( $p < 0.001$ ) than control subjects, while HDL cholesterol was lower ( $p < 0.005$ ). Others variables such as total cholesterol and LDL cholesterol levels, and serum paraoxonase and arylesterase activities did not show significant differences between the control and case groups.

**Table 1** Demographic details of the study groups.

	Control	Cases
Total number (male/female)	203 (105/98)	126 (83/43)
Age (years)	60.0 ± 1.3	63.4 ± 1.5
Total cholesterol (mmol/L)	5.10 ± 0.08	5.18 ± 0.15
Triglycerides (mmol/L)	1.62 ± 0.09	2.00 ± 0.16*
HDL cholesterol (mmol/L)	1.65 ± 0.03	1.52 ± 0.03**
LDL cholesterol (mmol/L)	2.69 ± 0.07	2.62 ± 0.11
Fasting glycemia (mmol/L)	5.58 ± 0.11	6.41 ± 0.28***
Paraoxonase activity (nmol/min/L)	64.5 ± 44.5	58.9 ± 46.6
Arylesterase activity (nmol/min/L)	84.9 ± 40.8	80.6 ± 42.9

HDL: high-density lipoprotein.

LDL: low-density lipoprotein.

\*  $p < 0.05$ .\*\*  $p < 0.005$ .\*\*\*  $p < 0.001$ .**Table 2** PON 1 QR192 and LM55 genotype distribution and allele frequencies in the study groups.

	Control <i>n</i> (%)	Cases <i>n</i> (%)
<i>QR192 genotypes</i>		
QQ	116 (0.57)	77 (0.61)
QR	62 (0.31)	35 (0.28)
RR	25 (0.12)	14 (0.11)
<i>Alleles</i>		
Q	294 (0.72)	189 (0.75)
R	112 (0.28)	63 (0.25)
	$p = 0.525$ OR = 1.143 (0.799–1.634)	
<i>LM55 genotypes</i>		
LL	88 (0.43)	48 (0.38)
LM	98 (0.48)	69 (0.55)
MM	17 (0.08)	9 (0.07)
<i>Alleles</i>		
L	274 (0.67)	165 (0.65)
M	132 (0.33)	87 (0.35)
	$p = 0.610$ OR = 0.914 (0.656–1.273)	

### PON1 genotype distribution and allele frequencies

PON1 QR192 and LM55 genotypes and allelic frequencies of control and case groups are given in Table 2. Frequencies of QR 192, QQ, QR and RR genotypes among the cases were 0.61, 0.28 and 0.11, respectively; in the control group, they were 0.57, 0.31 and 0.12, respectively. With respect to the frequency of Q192 allele, it was of 0.75 in cases and of 0.72 in controls. Frequencies of LM 55, LL, LM and MM genotypes among the cases were 0.38, 0.55 and 0.07, respectively; in the controls, they were 0.43, 0.48 and 0.08, respectively. The frequency of L55 allele was 0.65 in cases and 0.67 in controls. There were not significant differences between the gene and alleles frequencies for the PON1 QR 192 and LM 55 polymorphisms in controls and patients. Variant QR 192 deviates significantly from H–WE ( $p < 0.05$ ) both in cases and controls, while variant LM 55 is in equilibrium in controls ( $p = 0.154$ ) but deviates significantly in cases ( $p < 0.05$ ) (data not shown).

### PON1 genotype and serum paraoxonase and arylesterase activity

Table 3 shows that PON1 QR 192 polymorphism had a major effect on serum paraoxonase activity, without effect on serum arylesterase activity. In both the control and patient groups, paraoxonase activity was significantly higher in the RR genotype and lowest in the QQ genotype. The QR variant had an intermediate activity between QQ and RR. PON1 LM55 polymorphism, also had major effects, but on both serum paraoxonase and arylesterase activities. In both control and case groups paraoxonase and arylesterase activities were significantly higher in the LL genotype and lower in the MM genotype, while the LM variant showed intermediate activities between LL and MM. All differences were significant with  $p < 0.05$ . There were not differences between the control and patient groups for both polymorphisms.

**Table 3** The relationship between QR192 and LM55 variants and paraoxonase and arylesterase activities.

PON 192 genotypes	Control			Cases		
	QQ	QR	RR	QQ	QR	RR
<i>N</i>	116	62	25	77	35	14
Paraoxonase activity (nmol/min/L)	44.5 ± 3.1 <sup>a</sup>	81.3 ± 4.6 <sup>b</sup>	106.0 ± 10.3 <sup>c</sup>	36.3 ± 3.3 <sup>a</sup>	84.6 ± 6.9 <sup>b</sup>	113.1 ± 16.0 <sup>c</sup>
Arylesterase activity (nmol/min/L)	85.5 ± 3.7 <sup>a</sup>	89.0 ± 5.7 <sup>a</sup>	70.8 ± 6.3 <sup>a</sup>	76.7 ± 7.8 <sup>a</sup>	88.8 ± 6.9 <sup>a</sup>	74.2 ± 11.0 <sup>a</sup>
PON 55 genotypes	Control			Cases		
	LL	LM	MM	LL	LM	MM
<i>N</i>	88	98	17	48	69	9
Paraoxonase activity (nmol/min/L)	83.8 ± 4.8 <sup>a</sup>	52.7 ± 3.9 <sup>b</sup>	22.1 ± 5.5 <sup>c</sup>	83.0 ± 7.8 <sup>a</sup>	47.5 ± 4.1 <sup>b</sup>	21.1 ± 8.4 <sup>c</sup>
Arylesterase activity (nmol/min/L)	92.5 ± 4.3 <sup>a</sup>	82.7 ± 3.9 <sup>b</sup>	54.7 ± 9.1 <sup>c</sup>	98.2 ± 6.1 <sup>a</sup>	71.1 ± 4.4 <sup>b</sup>	51.4 ± 15.0 <sup>c</sup>

Different letters means significant difference ( $p < 0.05$ ).

**Table 4** The relationship between QR192 and LM55 variants and lipid profile and glycemia.

PON 192 genotypes	Control		Cases	
	QQ	QR + RR	QQ	QR + RR
<i>N</i>	116	87	77	49
Cholesterol (mmol/L)	4.96 ± 0.13 <sup>a</sup>	5.17 ± 0.11 <sup>a</sup>	5.31 ± .22 <sup>a</sup>	5.0 ± 0.23 <sup>a</sup>
Triglycerides (mmol/L)	1.58 ± 0.11 <sup>a</sup>	1.71 ± 0.16 <sup>a</sup>	2.24 ± 0.24 <sup>b</sup>	1.93 ± 0.22 <sup>a b</sup>
HDL (mmol/L)	1.59 ± 0.04 <sup>a</sup>	1.73 ± 0.05 <sup>b</sup>	1.53 ± 0.04 <sup>a</sup>	1.48 ± 0.05 <sup>a</sup>
LDL (mmol/L)	2.71 ± 0.10 <sup>a</sup>	2.67 ± 0.09 <sup>a</sup>	2.73 ± 0.14 <sup>a</sup>	2.44 ± 0.14 <sup>a</sup>
Glycemia (mmol/L)	5.54 ± 0.15 <sup>a</sup>	5.58 ± 0.16 <sup>a</sup>	6.79 ± 0.43 <sup>b</sup>	6.06 ± 0.35 <sup>a</sup>
PON 55 genotypes	Control		Cases	
	LL	LM + MM	LL	LM + MM
<i>N</i>	88	115	48	78
Cholesterol (mmol/L)	5.13 ± 0.11 <sup>a</sup>	5.03 ± 0.12 <sup>a</sup>	5.06 ± 0.33 <sup>a</sup>	5.24 ± 0.16 <sup>a</sup>
Triglycerides (mmol/L)	1.85 ± 0.17 <sup>a</sup>	1.47 ± 0.09 <sup>b</sup>	1.66 ± 0.18 <sup>a</sup>	2.41 ± 0.25 <sup>c</sup>
HDL (mmol/L)	1.69 ± 0.06 <sup>a</sup>	1.64 ± 0.04 <sup>a</sup>	1.47 ± 0.06 <sup>b</sup>	1.54 ± 0.04 <sup>b</sup>
LDL (mmol/L)	2.64 ± 0.10 <sup>a</sup>	2.74 ± 0.10 <sup>a</sup>	2.50 ± 0.17 <sup>a</sup>	2.68 ± 0.15 <sup>a</sup>
Glycemia (mmol/L)	5.48 ± 0.14 <sup>a</sup>	5.63 ± 0.16 <sup>a</sup>	5.56 ± 0.20 <sup>a</sup>	7.08 ± 0.22 <sup>b</sup>

Different letters means significant difference ( $p < 0.05$ ).

### PON1 genotype and lipid profile and glycemia

The relationship between QR192 and LM55 variants and lipid profile and glycemia is shown in Table 4. Elevated concentrations of triglycerides in cases correlate with the QQ variant or with the presence of the M allele. But nevertheless, in controls M allele correlates with low triglycerides concentration. Controls with the R allele show higher HDL levels however cases do not. But both cases with LL variant and with the presence of the M allele had lower HDL levels than controls with all LM55 variants. With respect to serum glucose levels, these were elevated in cases with the QQ variant or with the presence of M allele. Cholesterol and LDL did not show variations in control and cases with any variant of both polymorphisms.

### Discussion

The heterogeneity of the Argentinean population, particularly in the metropolitan area, which results in cultural, socioeconomic, and ethnic diversities, may represent an interesting factor for epidemiological and biomarkers studies. It is important to take into account that large differences in frequency between ethnic populations are known in the PON1 genotype distribution which may be the reason for differences among studies.<sup>23</sup> Our data on risk factors of atherosclerosis (Table 1) indicated that in the overall sample, cases had higher serum triglycerides and glycemia, and lower HDL cholesterol than controls, though total cholesterol, LDL cholesterol, and PON1 paraoxonase and arylesterase activities did not show significant differences between both groups. The development of CAD is believed to be largely under genetic control.<sup>24</sup> Alterations in circulating in PON1 concentrations have been reported in CAD. Many case-control studies have addressed, in particular, the putative role of the polymorphisms in the PON1 gene

regarding the enzymatic activities.<sup>25</sup> Several studies found associations between one or more polymorphisms of the PON genes, but the strongest associations were found with PON1 Q192R and L55M polymorphisms.<sup>26</sup> PON1 has antioxidant, antiatherosclerotic and anti-inflammatory functions by inhibiting LDL oxidative modification and suppressing the differentiation of monocytes into macrophages, which is the first stage in the development of atherosclerosis. Furthermore, PON1 prevents the accumulation of oxidized LDL and stimulates cholesterol efflux from macrophages.<sup>24</sup> One molecular basis of the paraoxonase polymorphisms is a missense mutation in the coding region of PON1 resulting in a glutamine (Q)/arginine (R) substitution at codon 192.<sup>15</sup> The PON1 Q192 alloform hydrolyzes paraoxon much less efficiently than does PON1 R192, while the opposite is true in case of soman or sarin. PON1 Q192 is also more efficient at metabolizing oxidized HDL or LDL than PON1 R192.<sup>3</sup> Another coding region polymorphism, resulting in aminoacid substitution at position 55 Leu(L)/Met(M), has been associated with plasma PON1 protein levels, specifically with PON1 M55 being associated with a low plasma PON1 level. In QR192 variant an excess of homozygotes was detected, leading to a H-WE deviation both in cases and controls. This phenomenon can be due to the presence of subgroups that may lead to Wahlunds' effect, namely, if two or more subpopulations have different allele frequencies then the overall heterozygosity is reduced, even if the subpopulations themselves are in a H-WE. With respect to LM55 variant it can observe in cases but not controls a H-WE deviation. This may indicate that the polymorphism may be associated with the disease. According to our data, we have not found a statistically significantly different level of allele distribution of PON 192 and PON 55 genotypes between patients and controls (Table 2). Only a slight correlation with more cases with the allele 192Q and less with the 55L were found. These two polymorphisms were associated with paraoxonase activity, which increased, as we corroborated



in CAD patients and healthy population (Table 3) in the order of QQ < QR < RR genotype in the PON1 192 polymorphism and MM < ML < LL genotype in the PON1 55 polymorphism. In both patients and controls, 192 QQ and 55 MM homozygotes have the lowest PON1 activity and 192RR and 55LL the highest. With respect to arylesterase activity, the Q192R polymorphism did not show any influence in cases and controls, but this activity increased in the order MM < ML < LL due to the L55M polymorphism in both populations, having the 55MM homozygote the lowest and the 55LL the highest. These results accord with other studies.<sup>26</sup> Only one article<sup>27</sup> reported that the 192R allele decrease arylesterase activity, although to a lesser extent to that of paraoxonase activity. Our results support the idea that lipid protection against oxidation by PON1 may be reduced in CAD patients because of lower enzyme activity as reported by Bayrak et al.,<sup>28</sup> Indeed, triglycerides were higher in cases with the 192QQ genotype or the 55M allele, though the decrease in PON1 activity was not manifested in cholesterol, LDL and HDL in our population of cases. In our study, the decrease of HDL observed in cases L55M with respect to controls is not due to the less PON1 activity because it was observed in patients with any of the three genotypes. The increased HDL concentration observed in controls with the 192R allele and the reduced triglycerides concentration in controls with the 55M allele are not explained considering only PON1 activity. There is controversy about the influence of the several PON1 genetic polymorphisms on CAD. In a recent review, Abelló et al.,<sup>26</sup> aim to update and clarify the scientific evidence available on PON enzymes and their roles in CAD. They found that the strongest correlation resides in a protective role of the 192Q allele and the negative effect of the 192R allele. Nevertheless some manuscripts reported that the 192Q allele was more frequent in CAD patients. The 55M allele of PON1 has also been associated with CAD but others suggested that the 55M allele of PON1 has a protective effect against CAD.<sup>29</sup> In spite that diabetic subjects were excluded for the study, after the biochemical determinations, those presenting fasting glucose serum values up to 7.3 mmol/L were included. We found in cases higher glycemia concentrations in 192QQ homozygotes as compared with homo- and heterozygous carriers of the mutant R allele and in homo and heterozygous carriers of the M allele as compared with 55LL homozygotes, correlating with low activities of paraoxonase for the Q192R genotype and of paraoxonase and arylesterase for the L55M genotype. These results are consistent with the greater antioxidant efficiency attributed to the R allele as insulin resistance is affected by oxidative stress<sup>30</sup> while polymorphism at position 55 (LM55) has also been shown to have an effect on PON1 concentrations and activities.<sup>19</sup> As a consequence the increased amount of ROS and oxidized LDL-C synergize to impair insulin signaling.<sup>31</sup> Then, PON1 polymorphisms could be associated with the two hyperglycemic generators in glucose metabolism, impaired glucose utilization for skeletal muscle and uninhibited hepatic glucose output, both insulin-mediated pathways and sensitive to oxidative stress.<sup>30</sup> In this work patients are not diabetics and it is very stochastic that PON1 activity was decreased by protein glycosylation, as other authors had argued,<sup>12,32,33</sup> based on several works that report that glycation of proteins including enzymes may decrease their activities in diabetes.<sup>34,35</sup> Our results suggest on the

contrary that primary low PON1 activities has high probability to determine the higher levels of blood glucose observed in subjects with a cardiovascular event. Metabolic syndrome is a set of common metabolic disorders that is strictly linked to CAD. Low-grade chronic inflammation, characterized by the production of abnormal cytokines such as IL-6, TNF- $\alpha$ , IL-1, leptin and resistin, is associated with insulin resistance in the liver. These factors inhibit insulin signaling in hepatocytes by activating the suppressor proteins of cytokine signaling, several kinases such as JNK, IKK- $\beta$  and PKC and protein tyrosine phosphatases such as PTP1B and PTEN that in turn impair insulin signaling at the insulin receptor and insulin receptor substrate level. Hepatic insulin resistance in turn causes impaired suppression of glucose production by insulin in hepatocytes leading to hyperglycemia. Our results suggest that the decrease in antioxidant protection due to the lower activity of 192QQ genotype and 55M allele would increase inflammatory cytokines capable to increase blood glucose generated by peripheral insulin resistance, causing the evolution of the atherosclerotic plaque.<sup>36-38</sup> In spite of the fact of the several limitations of the present study such as the relatively small size of the cohorts, and that the PON1 protein concentration, the insulin and the glycosylated hemoglobin were not measured, it constitutes a strong preliminary report in patients, about the contribution of PON1 polymorphism on the variations in glycemia associated with tryglyceridemia and CAD, enriched besides, for the heterogeneity of Buenos Aires population. It is known the association of diabetes with the bad CAD prognosis, but intrinsic metabolic mechanisms are in discussion. Our findings contribute to put in agenda the role of PON1 polymorphisms related with glucose metabolism.

## Conclusions

Increase triglycerides observed in cases confirm the relationship between variations in PON1 activities and lipid metabolism described by other works. PON1 low activities, determined by genetic polymorphism, result in a decrease in anti-inflammatory and antioxidant protection in CAD, characterized by increased levels of cytokines, which would determine an increase in blood glucose. The concomitant increase in triglycerides and blood glucose contribute to the aggravation of atherosclerotic disease and CAD.

## Ethical responsibilities

**Protection of people and animals.** The authors state that for this investigation have not been performed experiments on humans or animals.

**Confidentiality of data.** The authors declare that they have followed the protocols of the workplace on the publication of patient data.

**Right to privacy and informed consent.** The authors have obtained the informed consent of patients and/or subjects referred to in Article consent. This document is held by the corresponding author.

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## Conflict of interest

The authors declare no conflict of interest.

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