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Interaction between metabolic syndrome and PON1 polymorphisms as a determinant of the risk of coronary artery disease

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Abstract The enzyme serum paraoxonase plays an important role in antioxidant defences and prevention of atherosclerosis. Metabolic syndrome (MS) is a clinical condition associated with increased oxidant stress and cardiovascular mortality. Two common polymorphisms of serum paraoxonase, PON1 $Leu_{55}Met$ and $Gln_{192}Arg$, have been postulated to modulate the cardiovascular risk. We studied 915 subjects with angiographic documentation: 642 subjects with coronary atherosclerosis and 273 with normal coronary arteries. Two hundred and twenty-four subjects met the diagnostic criteria of MS. We found a significant interaction between MS and both the PON1 polymorphisms in determining the risk of coronary artery disease ($P<0.05$ by likelihood-ratio test). The 55Leu and the 192Arg alleles, associated with reduced protection against lipid peroxidation, were associated with coronary artery disease only in the MS subgroup. Subjects with MS and both 55Leu and 192Arg alleles had significantly increased risk (OR=9.38 with 95% CI=3.02–29.13 after adjustment by multiple logistic regression) as compared to subjects without MS and with 55Met/Met-192Gln/Gln genotype. No increased risk was found for subjects with MS and the 55Met/Met-192Gln/Gln genotype. This study highlights a potential example of genetic (paraoxonase polymorphisms)-clinical (MS) interaction influencing cardiovascular risk.

Key words PON1 polymorphisms • Metabolic syndrome • Coronary artery disease

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Introduction

Atherosclerosis is a complex disease, in which oxidative stress is recognised to play a prominent role [1]. Oxidised low-density lipoproteins (LDL) are thought to cause endothelial injury and foam-cell formation [2]. Recently, several genes that could be involved in oxidative stress and/or in antioxidant defences have been investigated in cardiovascular disease. Among these, the paraoxonase (PON) gene family holds a place of particular importance [3]. The PON gene cluster, located on chromosome 7q21.3–22.1, contains at least 3 members with about 65% similarity at the aminoacid level (PON1, PON2 and PON3) [4, 5]. PON1, which codes for serum paraoxonase, is the most extensively investigated. Serum paraoxonase (PON1) is a 45-kDa enzyme, synthesised by the liver and bound to high-density lipoproteins (HDL);

it is a calcium-dependent serum esterase that originally was known for its ability in protecting against organophosphate compounds, like paraoxon. In the last decade, serum paraoxonase has been demonstrated to be a major contributor to the antioxidative properties of HDL, partially explaining the protective role of HDL against atherosclerosis [6–10]. Furthermore, studies on human PON1 activity and on knock-out or transgenic mice have suggested PON1 as a determinant of the risk of atherosclerosis [11, 12]. PON3 has been identified as a lactonase associated with HDL and could co-operate in the prevention of LDL oxidation [13]. PON2 also has antioxidant properties, but unlike PON1 and PON3, which are expressed primarily in the liver, it is ubiquitously expressed, especially in endothelial and human aortic smooth muscle cells [14, 15].

Two common missense polymorphisms have been identified in the PON1 gene, $Leu_{55}Met$ ($L_{55}M$) and $Gln_{192}Arg$ ($Q_{192}R$) [4, 16, 17] and recent structural studies support the functional roles of these variations [18]. The HDL particles from 55 Met/Met and 192 Gln/Gln individuals have been demonstrated to be most effective in protecting LDL from oxidative modification [19]. The alleles less effective in protecting from oxidative stress, 55 Leu and the 192 Arg, have been associated with an increased risk of vascular disease in some clinical studies [20–35], but not all [36–49]. A recent meta-analysis showed no significant association between 55 Leu allele and coronary heart disease, whereas a weak, but statistically significant increased risk (OR=1.12 with 95% CI=1.07–1.16) was found for the 192 Arg allele [50]. The PON1 192 Arg/Arg homozygosity has been associated with an increased mortality in women in one study in a Danish population [51], whereas an opposite result with the 192 Arg allele associated with enhanced longevity was found in two very elderly European (Italian and Irish) populations [52]. About the latter result, the authors pointed out that the 192 Arg allele shows a higher enzymatic paraoxon hydrolytic activity and thus could be advantageous in the metabolism of potentially toxic chemicals. Furthermore, many factors, both genetic and environmental, can influence paraoxonase activity and some authors suggested that PON1 phenotype (activity and concentration) may be more important in determining cardiovascular risk than genetic polymorphisms [46, 53].

Metabolic syndrome (MS) is a widely diffuse condition associated with premature atherosclerosis and increased cardiovascular disease mortality [54, 55]. Notably, MS has been correlated with endothelial dysfunction and susceptibility to oxidative stress [56] and subjects with this clinical condition have been shown to present high concentrations of lipid peroxides and low paraoxonase activity [57].

In a previous study, we investigated the distribution of PON1 polymorphisms ($Leu_{55}Met$ and $Gln_{192}Arg$) and of PON2 $Ser_{311}Cys$ polymorphism in subjects with coronary angiographic documentation, highlighting an interaction between smoking and PON2 $Ser_{311}Cys$ polymorphism in determining the risk of myocardial infarction. In that work, no association was found between PON polymorphisms and coronary artery disease, or between PON1 polymorphisms and myocardial infarction [58].

The aim of this study was to re-analyse the distribution of three PON polymorphisms (PON1 $Leu_{55}Met$ and $Gln_{192}Arg$, and PON2 $Ser_{311}Cys$) in the context of MS within a larger population with coronary angiographic documentation. Our working hypothesis was that the clinical consequence of mutations that impair the antioxidant mechanisms could be particularly evident among subjects at increased risk of oxidative stress due to MS.

Materials and methods

Study population

The Verona Heart Project is an ongoing study aimed at finding new risk factors for CAD and MI in a population of subjects with objective angiographic documentation of their coronary vessels. Details of the enrolment criteria have been described elsewhere [59]. In the present study we used data from a total of 915 subjects, for whom analyses of PON gene polymorphisms and a clear-cut clinical definition of MS were available. Of these 915 subjects, 642 subjects had angiographically documented severe coronary atherosclerosis (CAD group), the majority of them being candidates for coronary artery bypass grafting.

Two hundred and seventy-three out of the 915 subjects with angiographic documentation had normal coronary arteries (CAD-free), and had been examined for reasons other than potential CAD, mainly valvular heart disease. Controls were also required to have neither history nor clinical or instrumental evidence of atherosclerosis in vascular districts beyond the coronary bed. As the primary aim of our selection was to provide an objective and clear-cut definition of the atherosclerotic phenotype, subjects with non-significant coronary stenosis (<50%) were not included in the study. The angiograms were assessed by two cardiologists unaware that the patients were to be included in the study.

All participants came from the same geographical area (Northern Italy), with a similar socio-economic background. At the time of blood sampling, a complete clinical history was collected, including the assessment of cardiovascular risk factors such as obesity, smoking, hypertension and diabetes. According to recently revised criteria [54], patients were classified as having MS when at least three of the following conditions were present [60]: body mass index (BMI)≥30 kg/m^2; documented history of hypertension or blood pressure >140/90 mmHg; history of diabetes or fasting glucose >110 mg/dl; plasma triglycerides >150 mg/dl and HDL <40 mg/dl for males or <50 mg/dl for females.

The study was approved by our institutional review board. Informed consent was obtained from all the patients after a full explanation of the study.

Biochemical analysis

Samples of venous blood were drawn from each subject in the free-living state, after an overnight fast. Serum lipids, as well as other CAD risk factors, including high-sensitivity C-reactive protein (hs-CRP) were determined immediately after collection as previously described [59, 61]. Insulin was measured by an immunometric "sandwich" assay (Immulite 2000 Insulin from Diagnostic Products Corporation, Los Angeles, CA). To obtain an estimate of insulin resistance, we applied the homeostasis model assessment (HOMA) using the following formula: HOMA=fasting insulin (μUI/ml)xfasting glucose (mmol/l)/22.5 [62]. Plasma malondialdehyde (MDA) was measured by an improved HPLC method as previously described [61], using HPLC Gilson 305–805 (Gilson, Middleton, WI, USA).

Polymorphism analysis

Genomic DNA was prepared from whole blood samples by phenol-chloroform extraction and then genotyped according to a previously described multilocus assay protocol [63]. Briefly, each sample was amplified by two 33-cycle Multiplex Polymerase Chain Reactions (32 ng of genomic DNA each) and the PCR products were then hybridised to an array of immobilised, sequence-specific oligonucleotide probes. The colorimetric detection was based upon streptavidin-horseradish peroxidase.

Statistics

The estimated haplotype frequencies (EH) programme was used to determine maximum-likelihood estimates of disequilibrium between pairwise combinations of alleles.

Calculations were performed mainly with the SSPS 11.5 statistical package (SPSS Inc., Chicago, IL). Distributions of continuous variables in groups were expressed as means±standard deviation. Logarithmic transformation was performed on all skewed variables, including insulin, HOMA, hs-CRP and MDA. Hence, geometric means with 95% confidence interval are given for these variables. Quantitative data were assessed using the Student's *t*-test or by ANOVA with Tukey's *post hoc* comparison of the means. Associations between qualitative variables were analysed with the chi-square test. A value of $P<0.05$ was considered significant.

To assess the association among various genotypes and CAD, odds ratios with 95% confidence intervals (CIs) were estimated by univariate logistic regression analysis. The population was then stratified in subgroups on the basis of different PON genotypes and the presence/absence of MS. To provide separate odds ratios for each subgroup, two dummy variables were used, with subjects without MS and with 55Met/Met-192Gln/Gln combined genotypes used as the reference groups. The adjustment for other classical vascular risk factors was performed by including these covariates in a second set of multiple logistic-regression models.

Analysis for potential interactions in determining CAD risk between PON polymorphisms and MS were performed using a likelihood-ratio test (Stata Statistical Software, 8.0, 2003, Stata Corporation, College Station, TX, USA); this analysis was then adjusted for the other classical risk factors of CAD, not included in the definition of MS (i.e., age, sex, smoke, LDL-cholesterol, hs-CRP).

Results

The characteristics of the patients are summarised in Table 1. As expected, conventional risk factors were more prevalent in CAD patients than in subjects free of coronary disease. A substantial proportion of the study population presented characteristics compatible with the clinical definition of MS (224/915 subjects – 24.5%) and this condition was associated with a greatly increased risk of CAD (OR=3.85; 95% CI=2.51–5.89). The clinical characteristics and PON genotype frequencies among patients according to the presence or absence of MS are reported in Table 2. There was no significant difference in plasma levels of MDA, a marker of lipid peroxidation, between CAD and CAD-free subjects, nor between patients with or without MS.

In our population, the two PON1 polymorphisms were in strong linkage disequilibrium (D'=0.905). On the other hand, the D' values between the PON1 polymorphisms and the PON2 $Ser_{311}Cys$ were 0.549 for PON1 $Leu_{55}Met$ and 0.045 for PON1 $Gln_{192}Arg$.

The distributions of PON genotype frequencies were similar between the entire CAD population and the subjects without CAD (Table 1), as well as between subjects with or without MS (Table 2).

When the PON1 genotype distribution between CAD and CAD-free subjects was re-analysed stratifying the population into different groups on the basis of MS, an asymmetry was evident. In subjects without MS PON1 genotype frequencies were similar in the CAD and CAD-free subgroups, while in the MS group there were significantly more carriers of 55 Leu and 192 Arg alleles in the CAD than in the CAD-free subgroup (P=0.009 and P=0.017 by χ^2-test, respectively) (Table 3). Heterozygous and homozygous carriers of 55 Leu and 192 Arg allele with MS presented a progressively increased risk of CAD. Figure 1 shows the odds ratios for CAD estimated by univariate logistic regression analysis after stratification for the PON1 genotypes and MS, assuming subjects without MS and with the 55 Met/Met or 192 Gln/Gln genotype, respectively, as the reference group.

Analysing the potential interactions in determining the CAD risk, both interactions between MS and the two PON1 genotypes were significant, also after adjustment for the classical risk factors of CAD not included in the definition of MS – i.e., age, sex, smoke, LDL cholesterol,

Table 1 Characteristics of the study population, with or without CAD

Characteristics	CAD-free (*n*=273)	CAD (*n*=642)	*P*
Age, years	57.8±12.3	60.6±9.4	<0.001*
Male sex, %	68.5	81	<0.001#
BMI, kg/m^2	25.3±3.5	26.7±3.4	<0.001*
Hypertension, %	32.6	61.4	<0.001#
Smoking, %	42.5	68.6	<0.001#
Diabetes, %	5.2	15.8	<0.001#
Fasting glucose, mmol/l	5.47±0.80	5.80±1.49	<0.001*
Total cholesterol, mmol/l	5.54±1.06	5.79±1.12	0.002*
LDL cholesterol, mmol/l	3.56±0.93	3.87±0.97	<0.001*
HDL cholesterol, mmol/l	1.44±0.42	1.20±0.31	<0.001*
Triglycerides, mmol/l	1.49±0.67	1.98±1.12	<0.001*
Insulin, μIU/ml	13.1 (12.2–14.1)	13.2 (12.8–13.7)	0.748*
HOMA	3.16 (2.92–3.43)	3.37 (3.22–3.52)	0.984*
hs-CRP, mg/l	1.94 (1.67–2.25)	3.17 (2.88–3.48)	<0.001*
Creatinine, mmol/l	94.5±36.9	98.0±37.7	0.203*
MDA, μmol/l	0.67 (0.65–0.68)	0.68 (0.67–0.69)	0.092*
Metabolic syndrome, %	10.3	30.5	<0.001#
PON1 $Leu_{55}Met$ genotype, %			
Leu/Leu	36.6	36.9	
Leu/Met	46.9	50	0.377#
Met/Met	16.5	13.1	
PON1 $Gln_{192}Arg$ genotype, %			
Gln/Gln	49.5	50.8	
Gln/Arg	43.2	39.6	0.395#
Arg/Arg	7.3	9.6	
PON2 $Cys_{311}Ser$ genotype, %			
Ser/Ser	63	60.7	
Cys/Ser	34.4	33.8	0.160#
Cys/Cys	2.6	5.5	

*By *t*-test, #by χ^2-test

hs-CRP (for $Leu_{55}Met$: χ^2=4.53, *P*=0.0332 by likelihood-ratio test; for $Gln_{192}Arg$: χ^2=11.03, *P*=0.0009 by likelihood-ratio test).

Considering the linkage disequilibrium between the two PON1 polymorphisms, PON1 genotypes were compiled considering both polymorphisms and the association with CAD was determined (Fig. 2). No relation was found in subjects without MS (Fig. 2a), whereas in patients with MS an incremental association with the risk of CAD was observed: the lowest risk was associated with the 55Met/Met-192Gln/Gln genotype and the highest risk with the 55Leu/Leu-192Arg/Arg genotype (Fig. 2b).

We then defined three groups: the carriers of 55Met/Met-192Gln/Gln, the "isolated" carriers of 55 Leu allele with 192Gln/Gln (subjects with 55Leu/Leu-192Gln/Gln or 55Leu/Met-192Gln/Gln genotype), and the "concomitant" carriers of 55 Leu and of 192 Arg alleles (subjects with 55Leu/Leu-192Gln/Arg or 55Leu/Met-192Gln/Arg or 55Leu/Leu-192Arg/Arg or 55Leu/Met-192Arg/Arg genotype). Because of the low number of subjects involved, the "isolated" carriers of 192 Arg allele with 55Met/Met (subjects with 55Met/Met-192Gln/Arg or 55Met/Met-192Arg/Arg genotype) were not considered.

In the MS group, "concomitant" carriers of 55 Leu and of 192 Arg alleles presented an increased risk of CAD compared with 55Met/Met-192Gln/Gln subjects, even after multiple logistic regression adjustment for all the classical risk factors for coronary heart disease, including those in the definition of MS (OR 5.4 with 95%CI 1.16–25.29). Notably, among CAD subjects with MS, the carriers of 55Met/Met-192Gln/Gln genotype were significantly older (64.3±9.3 years) than carriers of 55 Leu and 192 Arg alleles (55 Leu "isolated" carriers: 58.0±9.0 years; "concomitant" carriers of 55 Leu and of 192 Arg

Table 2 Characteristics of the study population, with or without metabolic syndrome

Characteristics	Metabolic syndrome-free (*n*=691)	Metabolic syndrome (*n*=224)	*P*
Age, years	60±10.8	59.1±9.2	0.207*
Male sex, %	76.8	78.6	0.592#
BMI, kg/m^2	25.5±3.1	28.7±3.5	<0.001*
Hypertension, %	42.8	83.9	<0.001#
Smoking, %	59	66.5	0.049#
Diabetes, %	5.6	35	<0.001#
Fasting glucose, mmol/l	5.43±0.98	6.53±1.84	<0.001*
Total cholesterol, mmol/l	5.68±1.06	5.83±1.23	0.109*
LDL cholesterol, mmol/l	3.74±0.94	3.88±1.05	0.066*
HDL cholesterol, mmol/l	1.36±0.35	1.01±0.25	<0.001*
Triglycerides, mmol/l	1.59±0.79	2.58±1.29	<0.001*
Insulin, μIU/ml	12.7 (12.2–13.2)	15.0 (14.0–16.0)	<0.001*
HOMA	3.04 (2.91–3.18)	4.25 (3.94–4.59)	<0.001*
hs-CRP, mg/l	2.64 (2.39–2.9)	3.12 (2.71–3.59)	0.055*
Creatinine, mmol/l	95.7±38.4	100.7±34.2	0.092*
MDA, μmol/l	0.68 (0.67–0.69)	0.67 (0.66–0.69)	0.892*
CAD, %	64.5	87.5	<0.001#
PON1 Leu_{55}Met genotype, %			
Leu/Leu	36.7	37.1	
Leu/Met	49.8	46.9	0.573#
Met/Met	13.5	16	
PON1 Gln_{192}Arg genotype, %			
Gln/Gln	49.3	53.6	
Gln/Arg	42.3	35.7	0.183#
Arg/Arg	8.4	10.7	
PON2 Cys_{311}Ser genotype, %			
Ser/Ser	60.3	64.7	
Cys/Ser	35.3	29.9	0.307#
Cys/Cys	4.4	5.4	

*By *t*-test, #by χ^2-test

Table 3 Distribution of PON1 genotypes in CAD and CAD-free subgroups, stratified on the basis of the presence/absence of metabolic syndrome. Data are *n* (%)

	PON1 55 Leu/Leu	PON1 55 Leu/Met	PON1 55 Met/Met	*P**	PON1 192 Gln/Gln	PON1 192 Gln/Arg	PON1 192 Arg/Arg	*P**
No metabolic syndrome (*n*=691)								
CAD-free subjects (*n*=245)	93 (38)	117 (47.7)	35 (14.3)	0.721	113 (46.1)	113 (46.1)	19 (7.8)	0.312
CAD subjects (*n*=446)	161 (36.1)	227 (50.9)	58 (13.0)		228 (51.1)	179 (40.1)	39 (8.8)	
Metabolic syndrome (*n*=224)								
CAD-free subjects (*n*=28)	7 (25.0)	11 (39.3)	10 (35.7)	0.009	22 (78.6)	5 (17.9)	1 (3.5)	0.017
CAD subjects (*n*=196)	76 (38.5)	94 (48)	26 (13.2)		98 (50)	75 (38.3)	23 (11.7)	

*By χ^2-test

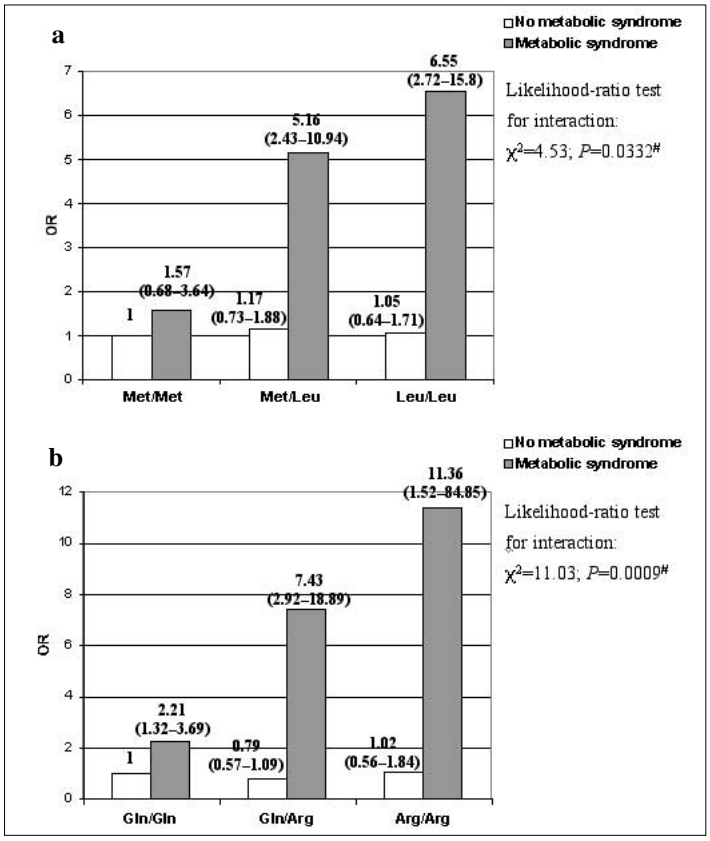
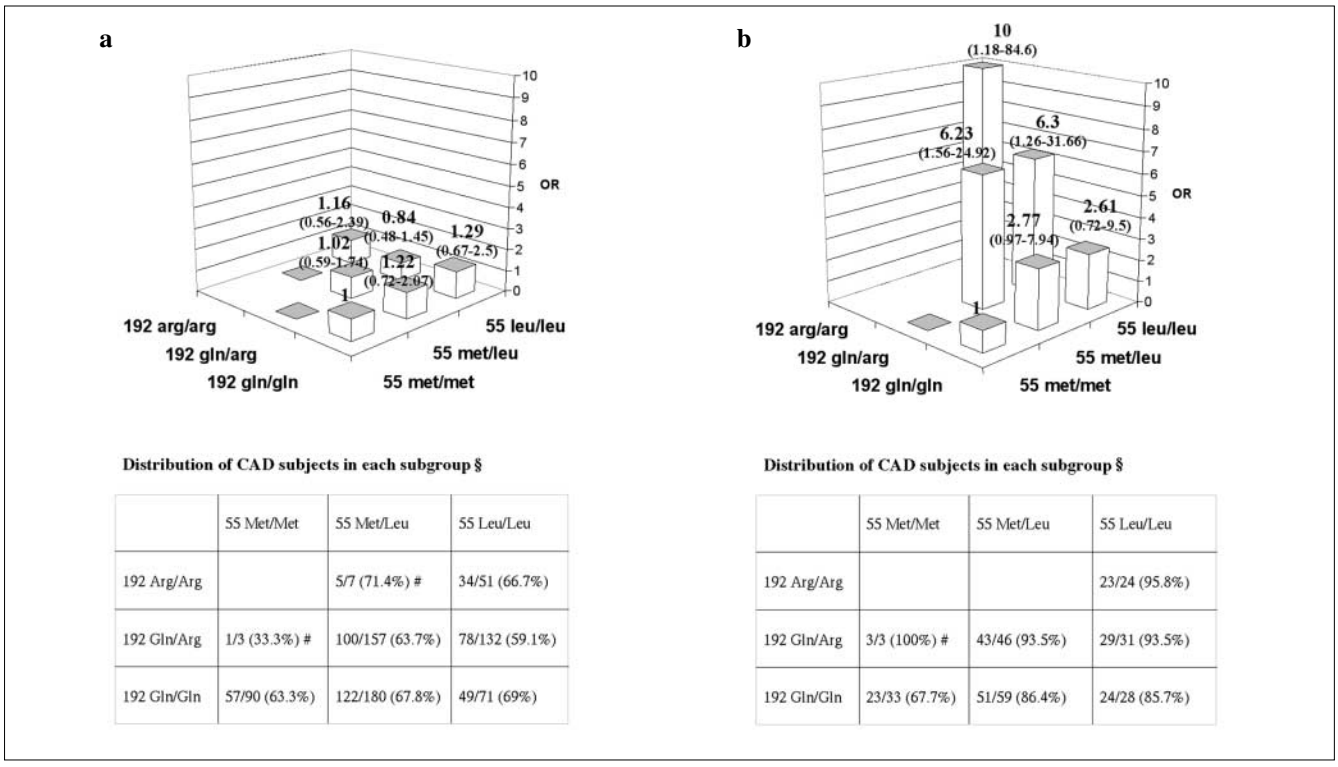


Fig. 1 Interaction between PON1 $Met_{55}Leu$ (**a**) or $Gln_{192}Arg$ (**b**) polymorphism and metabolic syndrome as determinant of CAD risk. Subjects without metabolic syndrome and with 55 Met/Met (**a**) or 192 Gln/Gln genotype (**b**), respectively, are considered as the reference groups; OR are presented with 95% CI and are calculated by univariate logistic regression analysis. #By likelihood-ratio test, considering the interaction between Leu allele carrier and metabolic syndrome, after adjustment for the other classical risk factors of CAD, that are not included in the definition of metabolic syndrome (age, sex, smoker, LDL cholesterol, hs-CRP)



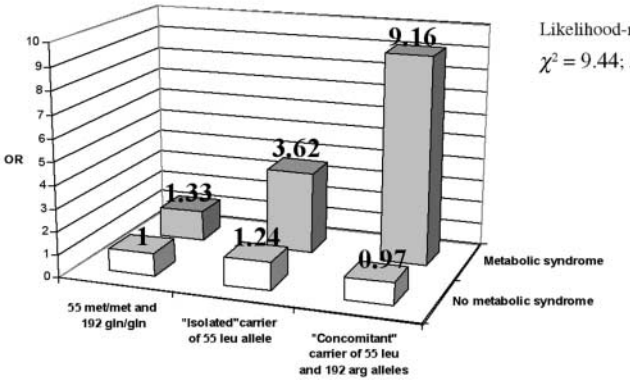
Distribution of CAD subjects in each subgroup §

	55 Met/Met	55 Met/Leu	55 Leu/Leu
192 Arg/Arg		5/7 (71.4%) #	34/51 (66.7%)
192 Gln/Arg	1/3 (33.3%) #	100/157 (63.7%)	78/132 (59.1%)
192 Gln/Gln	57/90 (63.3%)	122/180 (67.8%)	49/71 (69%)

Distribution of CAD subjects in each subgroup §

	55 Met/Met	55 Met/Leu	55 Leu/Leu
192 Arg/Arg			23/24 (95.8%)
192 Gln/Arg	3/3 (100%) #	43/46 (93.5%)	29/31 (93.5%)
192 Gln/Gln	23/33 (67.7%)	51/59 (86.4%)	24/28 (85.7%)

Fig. 2 PON1 combined genotypes and CAD risk in subjects without (**a**) and with (**b**) metabolic syndrome. Both in metabolic syndrome-free group and in metabolic syndrome group, subjects with 55 Met/Met and 192 Gln/Gln genotype are considered as the reference groups; OR are presented with 95% CI. §Fractions refer to the number of CAD subjects over total subjects in the class. #The analysis for CAD odds ratio was not performed because of the low number of subjects in the subgroup



Odds ratio of CAD with 95% CI ##

	55 Met/Met and 192 Gln/Gln (n=123)	"isolated" carrier of 55 Leu allele * (n=338)	"concomitant" carrier of 55 Leu and 192 Arg alleles ** (n=448)
No metabolic syndrome (n=688)	1 § (n=90)	1.24 (0.75-2.05) (n=251)	0.97 (0.60-1.56) (n=347)
Metabolic syndrome (n=221)	1.33 (0.57-3.14) (n=33)	3.62 (1.72-7.62) (n=87)	9.16 (3.62-23.21) (n=101)

Fig. 3 Interaction between PON1 combined genotypes and metabolic syndrome as a determinant of CAD risk. § Subjects without metabolic syndrome and with 55 Met/Met-192 Gln/Gln genotype are considered as the reference groups. # By likelihood-ratio test, considering the interaction between PON1 combined genotypes and metabolic syndrome in determining CAD risk, after adjustment for the other classical risk factors of CAD, that are not included in the definition of metabolic syndrome (age, sex, smoker, LDL cholesterol, hs-CRP). ## By univariate logistic regression analysis. *Subjects with 55Leu/Met-192Gln/Gln or 55Leu/Leu-192Gln/Gln genotype; **subjects with 55Leu/Met-192Gln/Arg or 55Leu/Leu-192Gln/Arg or 55Leu/Met-192Arg/Arg or 55Leu/Leu-192Arg/Arg genotype

alleles: 59.0±8.9 years; F=4.415, P=0.013 by ANOVA; P=0.010 and P=0.030, respectively, by Tukey *post hoc* comparison).

Figure 3 shows the odds ratios for CAD estimated by univariate logistic regression analysis after stratification for the PON1 combined genotypes and MS, assuming subjects without MS and with the 55 Met/Met-192 Gln/Gln genotype as the reference group. No significant increased risk of CAD was found for subjects with MS and 55Met/Met-192Gln/Gln genotype, whereas a highly significant increased risk of CAD was found for subjects with MS and carriers of 55 Leu and of 192 Arg alleles (this association remained after multiple logistic regression adjustment for the classical risk factors of CAD not included in the definition of MS; for 55 Leu "isolated" carriers: OR=3.19 with 95% CI=1.37–7.44; for "concomitant" carriers of 55 Leu and of 192 Arg alleles: OR=9.38 with 95% CI=3.02–29.13).

In our study population there were no significant differences of plasma lipids, glucose or insulin levels and HOMA score associated with these PON1 polymorphisms (data not shown). Similar results were also observed in the subgroups of subjects with or without MS. There was also no significant difference of MDA concentrations among PON1 genotypes (PON1 55 Met/Met 0.68 (0.66–0.71); Met/Leu 0.67 (0.66–0.69); Leu/Leu 0.68 (0.66–0.69) μmol/l; P=0.773 by ANOVA; PON1 192 Gln/Gln 0.68 (0.67–0.69); Gln/Arg 0.67 (0.66–0.69); Arg/Arg 0.68 (0.64–0.71) μmol/l; P=0.843 by ANOVA). We did not find any differences considering the stratification on the basis of PON1 genotypes and MS (data not shown).

In reference to PON2 $Ser_{311}Cys$ polymorphism, no association with CAD or interaction with MS in determining the risk of CAD was found (data not shown).

Discussion

The association of PON polymorphisms with cardiovascular events is a controversial issue. The PON1 55 Leu and 192 Arg isoforms have been associated to an increased risk of CAD in some clinical studies [20–35], but not all [36–49]. This controversy was addressed by a recent meta-analysis including about 24 000 subjects [50], which showed no significant association between $Leu_{55}Met$ and CAD. On the other hand, a weak but significant association (OR=1.12 with 95% CI=1.07–1.16 for the 192 Arg allele) was found between the $Gln_{192}Arg$ and CAD, defined "of uncertain importance" by the authors. Meta-analysis results should be cautiously interpreted taking into account the high heterogeneity of studied populations and clinical outcomes; nevertheless, the studies that showed the greatest positive association between PON1 polymorphisms and vascular risk were those with high-risk populations, mainly in subjects with diabetes [20, 24, 28, 29, 32, 35].

The Verona Heart Project population has some advantages for genetic association studies: the objective definition of CAD phenotype by angiography and the possibility to dissect many clinical characteristics, including those that define the MS.

As in our previous study [58], we found no significant association between PON polymorphisms and CAD in the entire study population. However, after stratification of CAD patients in subgroups according to the presence or absence of MS, the PON1 polymorphisms showed a significant interaction with MS in determining the risk of CAD. MS is a clinical condition of high cardiovascular risk, which presents an enhanced propensity to develop premature atherosclerosis and is associated with high levels of lipid peroxides [55, 56]. Serum paraoxonase contributes to the prevention of LDL oxidation and lipid peroxidation [19]. These considerations prompted us to derive a biologically rational hypothesis of interaction between MS and PON1 in the mechanisms of atherogenesis.

In our analyses, PON1 55 Leu, and particularly the 192 Arg allele, known to reduce protection against lipid peroxidation [19, 64, 65], were associated with CAD only in the high-risk subgroup of MS subjects. In this context, it may be biologically plausible to speculate that the PON1 variants less effective in protecting LDL from oxidation might be more detrimental in a clinical condition with high oxidative stress. Conversely, subjects with the 55 Met/Met and 192 Gln/Gln genotype, known to be most effective at protecting LDL from oxidation, appeared to be protected from the detrimental cardiovascular effects of MS. Indeed in our study population subjects with MS and carriers of 55 Met/Met and 192 Gln/Gln genotype did not have a significant increased risk of CAD (OR=1.33 with 95% CI=0.57–3.14, as compared to subjects without MS and with 55 Met/Met and 192 Gln/Gln genotype), whereas a severely increased risk was found for subjects with MS carriers of 55 Leu and of 192 Arg alleles also after adjustment for the other CAD risk factors (for 55 Leu "isolated" carriers: OR=3.19 with 95% CI=1.37–7.44; for "concomitant" carriers of 55 Leu and of 192 Arg alleles: OR=9.38 with 95% CI=3.02–29.13). Notably among CAD subjects with MS, the carriers of 55 Leu and 192 Arg alleles were significantly younger than carriers of the 55 Met/Met/192 Gln/Gln genotype, further supporting the hypothesis of predisposition to early atherosclerosis in subjects with these "risk-susceptible genotypes", if a "high risk condition" (MS) is concomitant.

As in our previous work [58], we did not find a significant relationship between PON1 genotypes and MDA concentrations. Nevertheless it should be remarked that plasma levels of MDA are not an ideal marker of lipid peroxidation; perhaps this parameter could be inappropriate for detecting a relationship between paraoxonase and oxidative stress. It is worth noting that in our study population there was also no difference of MDA concentrations between CAD and CAD-free subjects, nor between patients with or without MS.

There are controversial observations about an association between PON1 polymorphisms and glucose and lipid metabolism [23, 29, 38, 66–68]. We did not observe any significant relation between PON1 genotypes and glucose, insulin and lipids in our population. Similarly, we did not find any association between PON1 genotypes and MS.

Our study has several limitations. First, we did not measure PON1 activity. On the other hand, it should be emphasised that PON activity is substrate dependent, and the classical measurement of PON1 activity *vs*. paraoxon is not strictly indicative of PON1 activity *vs*. lipid peroxides. Indeed the paraoxon hydrolytic activity has been shown to be higher in 55 Leu/Leu and 192 Arg/Arg subjects and lower in 55 Met/Met and 192 Gln/Gln individuals [22], whereas the HDL particles from 55 Met/Met and 192 Gln/Gln have been demonstrated to be most effective at protecting LDL from oxidative modification [19]. Other limitations are the cross-sectional design and the relatively small sample size of the MS group, especially in the CAD-free subgroup. Thus, our observations need to be confirmed by further studies, preferably with a prospective design.

In conclusion this study highlights a potential example of genetic (PON1 polymorphisms)–clinical (MS) interaction in determining CAD risk. If confirmed, it may provide the basis for the identification of subjects particularly susceptible to MS-associated cardiovascular risk. Our observations may be particularly important considering the "epidemic" prevalence of MS (24–42% of the US general population [69]), as well as the high prevalence of risk-susceptible PON1 alleles. In fact, in our study population only 13.4% of subjects had the "fully protective" 55 Met/Met-192 Gln/Gln genotype. With respect to this point, it is tempting to speculate that the 55 Leu and 192 Arg PON1 variants could have other benefits, contributing to the high prevalence in the general population, whereas their harmful effects may have emerged only recently in human evolution, concomitantly with the dramatically rapid life-changes that are related with modern development (i.e., the exponential increase of previously unusual conditions, such as obesity and MS).

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